

Molekulares Screening in der mikrobiologischen Diagnostik



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Bellinzona

- Diagnostik
 - Molekulare Diagnostik vs. Kultur und biochemischen Methoden
 - 16S RNA
 - *gyrB*, *rpoB*, *hsp60*
- Screening
- Pilze
 - *Candida*
 - *Aspergillus*
 - FISH

Diagnostik

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Molekulare Diagnostik oder Kultur?

Molekulare Diagnostik

- Sehr empfindlich
- Schnell
- Weniger empfindlich auf Antibiotika-Behandlung
- Falsch positive Ergebnisse
- Kontaminationsgefahr

Kultur

- Empfindlich
- Langsam
- Meistens nicht zuverlässig nach Antibiotika-Behandlung
- Falsch negative Ergebnisse
- Kaum Kontaminationen

- Standardisierung
 - Ein Primer-Set wird kaum alle Bakterien amplifizieren
 - Positive und negative Kontrollen
- Kontaminationsgefahr: „Good molecular diagnostic practices (GMDP)“
(Sontakke et al. J Microbiol Methods 2009;76)

- Dedicated pre-PCR and post-PCR rooms with positive pressure and/or self-sustained facility
- Education of personnel for gowning and glove wearing
- Control of reagents through screening
- Dedicated equipment
- Iodine scrub before withdrawal of blood or collection of sample during surgery



16S rDNA

- Endocarditis
 - 16S rDNA PCR schneller und empfindlicher als Kultur (75 vs. 25)*
 - mehr Fälle diagnostiziert als mit Kulturen**
- ZNS
 - 16S rDNA PCR verbessert die Sensitivität der Diagnose in Patienten mit negativen Kulturen
- Andere Infekte
 - *M. tuberculosis*, *S. pneumoniae* und *Actinomyces neuii* mit PCR nachgewiesen – negative Kulturen nach AB-Behandlung***

* Marin et al. Medicine 2007;86; Voldstedlund et al. APMIS 2008;116

** Gauduchon et al. J Clin Microbiol 2003;41

*** Levy et al. Eur Heart J 2006;27.



16S rDNA als „Gold Standard“?

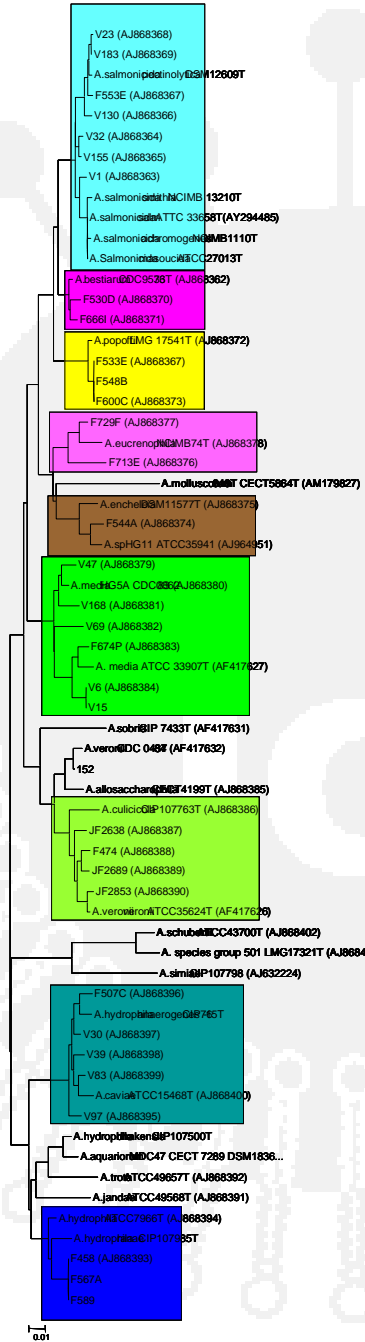
Identifikation von *Staphylococcus* spp. mit verschiedenen Methoden
 (% Übereinstimmung; $\Sigma \leq 100$)

		16S rDNA			
		<i>aureus</i>	<i>epidermidis</i>	<i>haemolyticus</i>	<i>hominis</i>
PHOENIX	<i>aureus</i>	93.3	.0	5.8	1.9
	<i>epidermidis</i>	1.9	74.2	.0	14.8
	<i>haemolyticus</i>	.0	.0	82.7	7.4
	<i>hominis</i>	.0	8.1	1.9	57.4
API	<i>aureus</i>	84.9	.0	1.9	.0
	<i>epidermidis</i>	.0	88.7	.0	10.4
	<i>haemolyticus</i>	3.8	.0	84.6	3.7
	<i>hominis</i>	.0	3.2	1.9	66.7

Alternativen: andere Gene



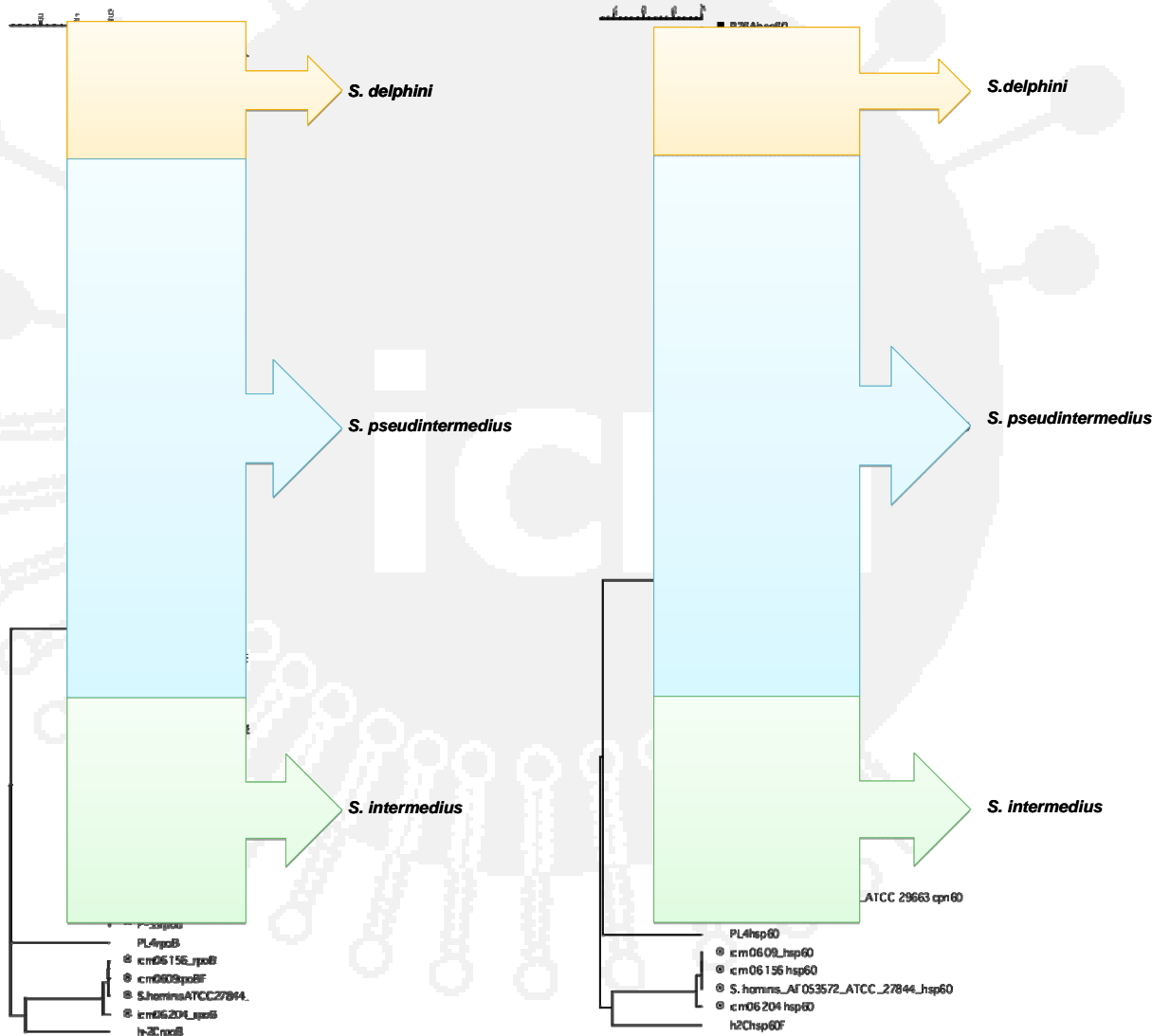
Aeromonas spp.



Unrooted phylogenetic tree based on gyrB gene sequencing (UPGMA)



Differenzierung von Isolaten der *S. intermedius*-Gruppe



Unrooted phylogenetic tree based on *rpoB* and *hsp60* gene sequencing (UPGMA)

*Fasola et al., submitted



CLSI: MM18-A

- *Interpretive Criteria for Identification of Bacteria and Fungi by DNA Target Sequencing; Approved Guideline (MM18-A) 2008*
 - „It is the aim of these guidelines to provide interpretive criteria for microorganism identification with an emphasis on 16S rRNA gene for bacteria and ITS regions for fungi. Alternative DNA targets are addressed when appropriate.“ [LABMEDICINE. 2010;41(2): 116-117; February 2010]

Screening

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MRSA:

to screen or not to screen...

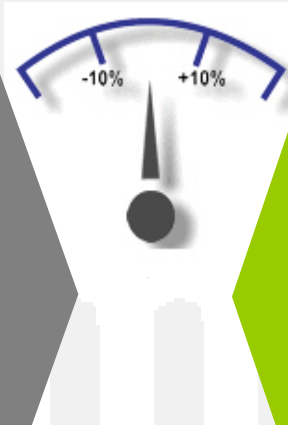
- **PRO: Chaberny, 2008; Huang, 2006**
 - deutliche Reduktion nosokomialer MRSA Infektionen nach Einführung eines Screeningprogramms
- **CONTRA: Habarth, 2008**
 - keine Reduktion der MRSA Infektionen
- **TATSACHE:**
 - 1 von 10 Patienten im Krankenhaus wird infiziert



PCR versus Kultur: MRSA

pro

- **geringer Zeitaufwand:** Stunden versus Tage
- **effektives Hygienemanagement:** schnelleres Ergebnis, frühzeitiges Einleiten weiterer Schritte, z.B. Isolation
- **geringere MRSA Übertragungsrate**
- **höhere Sensitivität:** chromogene Kultur schlechter als PCR



contra

- **geringere Spezifität:** angereicherte Kultur ist der Goldstandard
- **höhere Kosten pro Test**
- ein schnelleres Ergebnis hat **keinen zusätzlichen Nutzen**

MRSA screening

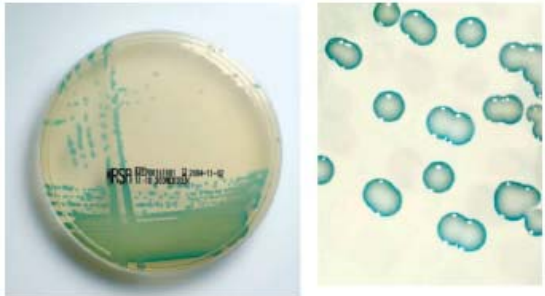


FIG. 1. MRSA on MRSA ID medium.

Chromogenes agar...



1. Insert swab into Elution Reagent vial and break at score



2. Vortex and dispense Sample into port 5



3. Dispense Reagent 1 into port 1



4. Dispense Reagent 2 into port 2



5. Insert cartridge and start assay

... oder GeneXpert System?





Anderere Screening tests

- BD GeneOhm MRSA Assay
- Roche LightCycler[®] MRSA Advanced Test
- GeneXpert Cepheid Real-time PCR für
 - MRSA/SA SSTI
 - MRSA/SA BC
 - VRE
 - *C. difficile*
 - Gruppe B *Streptococcus*



MRSA: Screeningsysteme im Vergleich

	MRSA Advanced Test	GeneOhm MRSA Assay	Cepheid GenXpert MRSA	chromogene Kultur
Relative Sensitivität*	92.3%	93.6%	94.3%	80.5%
Relative Spezifität*	98.9%	94.3%	93.2%	99.9%
PPV	94.0%	75.5%	73.0%	99.4%
NPV	98.5%	98.7%	98.8%	96.5%

* Kulturanreicherung = Goldstandard

- MRSA

- *mecA*: altered penicillin binding proteins (PBPs)
- HA-MRSA:
 - SCCmec Typ I-III
- CA-MRSA:
 - PVL (?)
 - SCCmec Typ IV und V



- Typisierung von *S. aureus*
 - PFGE
 - *Staphylococcus* protein A gene (spa)
- ICM Bellinzona:
 - Seit 2008 spa-typing für epidemiologische Untersuchungen wöchentlich durchgeführt
 - Profil mit Ridom Software bestimmt (<http://SpaServer.ridom.de>)



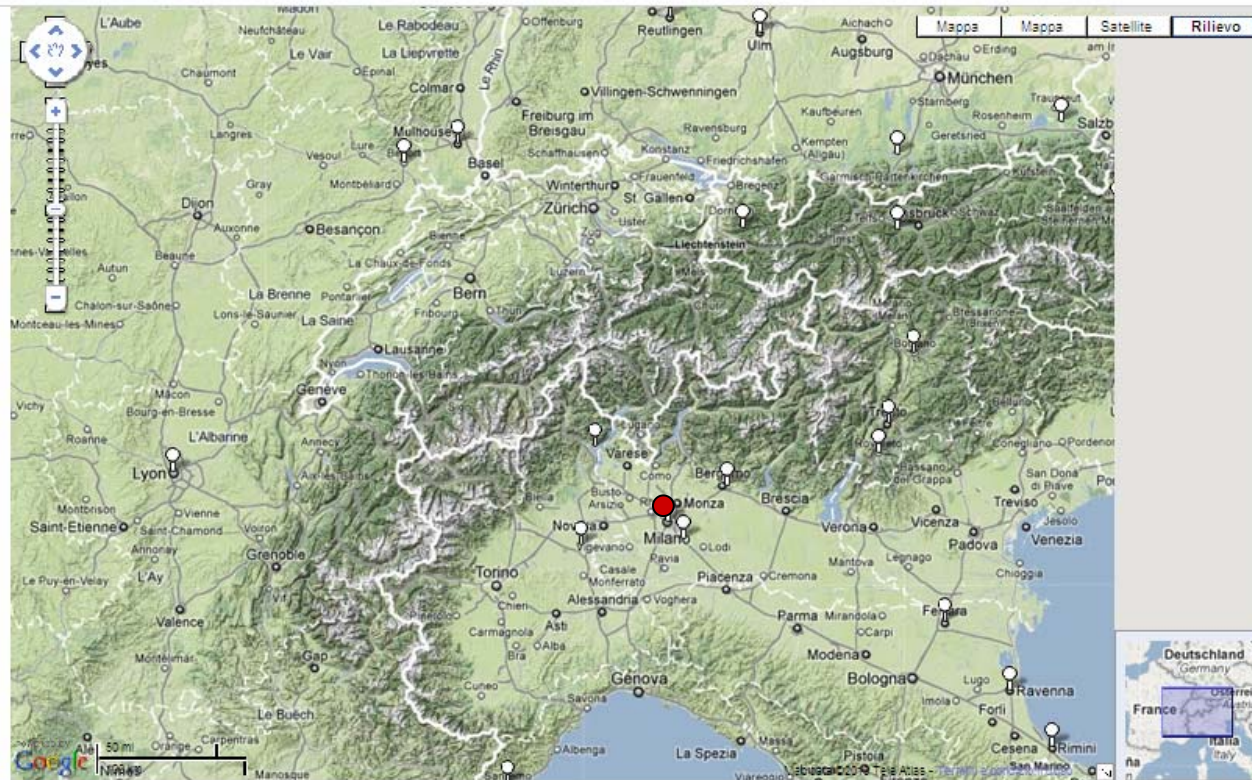
Spa Profile im Kanton Tessin

<i>Typ</i>	<i>Tessin</i>	<i>Weltweit</i>
t515	34%	0.37%
t1251	12%	0.14%
t002	11%	5.9%
t3138	7%	0.06%
t008	5%	7.13%
t3130	4%	0.04%



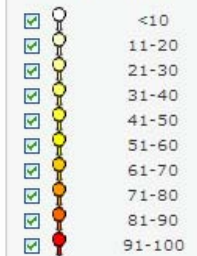
Staphylococcal Reference Laboratories Maps (SRL-Maps)

Spatialepidemiology.net | SRL-Maps



- [Home](#)
- [Instructions](#)
- [Isolate Database](#)
- [spa type Google Map](#)
- [spa type Google Earth](#)
- [Acknowledgements](#)

All Isolates Key (%total isolates):



Click on the SRL marker to view details of isolates.

When displayed you can also view other SRL's where a particular *spa* type is found.

IT25 *spa* Type Summary

[Click to return to European view.](#)

No. isolates	<i>spa</i> Type	Pattern	other laboratories (no. isolates elsewhere with same <i>spa</i> Type)	Action
2	t041	r26r30r17r34r17r20r17r34r17r20r17r12r17r16	37(70)	click to view on map
1	t084	r07r23r12r34r34r12r12r23r02r12r23	71(89)	click to view on map
1	t2961	r08r16r02r16r34r13r13r13r16r34	0	-
1	t012	r15r12r16r02r16r02r25r17r24r24	68(85)	click to view on map
1	t030	r15r12r16r02r24r24	11(21)	click to view on map
1	t515	r26r23r23r13r23r31r29r17r31r29r17r25r16r16r28	9(11)	click to view on map
1	t032	r26r23r23r13r23r31r29r17r31r29r17r25r17r25r16r28	61(141)	click to view on map



Spa-typing

“However, the finding that MRSA spa types occur mainly in geographical clusters has important implications for the control of MRSA, because it indicates that a limited number of clones are spreading within health care networks, which means that MRSA is mainly spread by patients who are repeatedly admitted to different hospitals”

Grundmann et al. (2010) Geographic Distribution of *Staphylococcus aureus* Causing Invasive Infections in Europe: A Molecular-Epidemiological Analysis. PLoS Med 7(1): e1000215.

Pilze

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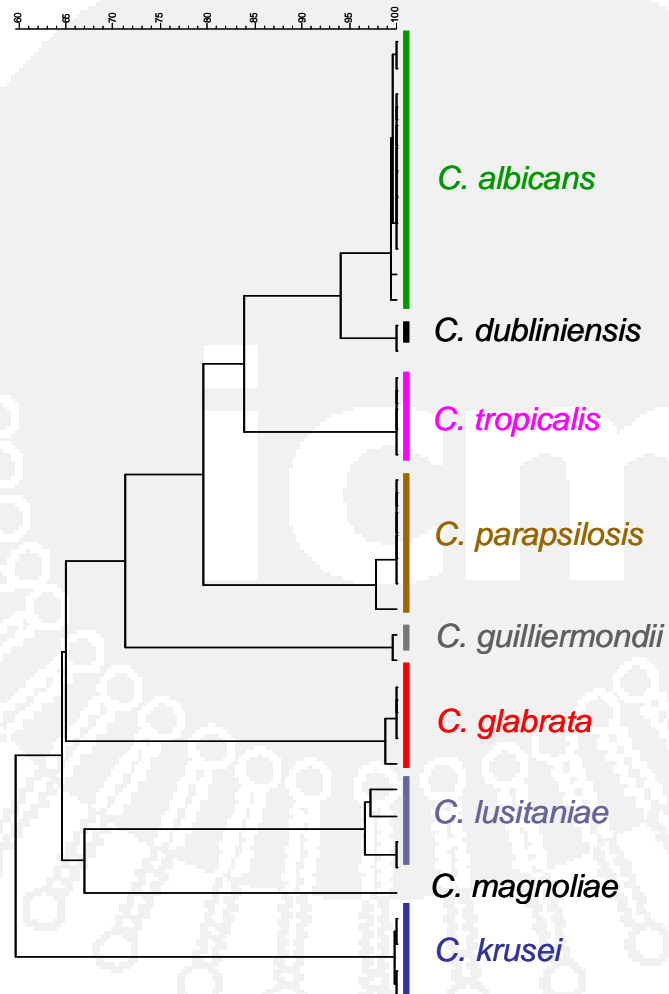


Identifikationsmethoden

- Kulturmethode
- Molekulare Methoden
 - ITS1, ITS2
 - Barcoding
 - FISH

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ITS-Sequenzierung: Candida



Phylogenie von *Candida* spp.
anhand der ITS1-Sequenzen



Barcoding

All Fungi Barcoding

About Us

All Fungi Barcoding

International Subcommittee on Fungal Barcoding

All Fungi Barcoding > About Us

- About Us
- Campaigns
- Meetings
- Fungal Barcoding Publications**

Welcome

The purpose of this website is to provide up-to-date information on fungal barcoding and to facilitate communication and the development of collaboration among researchers interested in this topic.

DNA barcoding is the use of short, standardized segments of the genome for identification of species in all the Kingdoms of Life. The goal of this All Fungi Barcoding site is to promote the DNA barcoding of the Kingdom Fungi and other fungus-like organisms.

Fungi are a large, diverse and economically important group of organisms. Estimates of the actual number of fungal species vary widely, from about 1.5 million to 13.5 million, with fewer than 100,000 now known. Some fungi have relatively complex and conspicuous morphologies, but others have very simple morphologies; many fungi have been detected using DNA sequences, but have never been seen. Because of their cryptic nature, fungal species are particularly suitable for DNA-based identification.

Fungal barcoding provides unique opportunities and challenges. The [International Subcommittee on Fungal Barcoding](#) (a subcommission of the International Commission on the Taxonomy of Fungi) has been established to promote and coordinate research on this topic. We welcome the ideas and participation of mycologists from all countries, working with any group of fungi and fungus-like organisms.

<http://www.allfungi.com/publications.php>

- Tiere
 - mitochondriale CO1 (*cox1*)
- Pflanzen
 - *cox1* (Rotalgen; Saunders 2005)
 - ITS und Chloroplastengene (intergenic spacer, *rbcL*; Kress et al. 2005; Chase et al. 2005; Newmaster et al. 2006)
- Protista
 - ssu rRNA (Scicluna et al. 2006)
- Pilze: Work in progress
 - *Trichoderma*: ITS (Druzhinina et al. 2005)
 - *Fusarium*: elongation factor 1 α (TEF) (Geiser et al. 2004)
 - *Penicillium*: *cox1* (Seifert et al. 2007)
 - *Aspergillus*: *cox1*?

- ITS
 - Die beste Region für die meisten Pilze
- Cytochrome c Oxidase 1 (CO1)
 - *Penicillium* als Modell (Seifert et al. PNAS 2007;104)
- Weitere Gene
 - Calmodulin, β -Tubulin, IGS

NOTES

Differentiation of *Candida albicans* and *Candida dubliniensis* by Fluorescent In Situ Hybridization with Peptide Nucleic Acid Probes

KENNETH OLIVEIRA,¹ GERHARD HAASE,² CLETUS KURTZMAN,³
JENS JØRGEN HYLDIG-NIELSEN,¹ AND HENRIK STENDER^{1*}

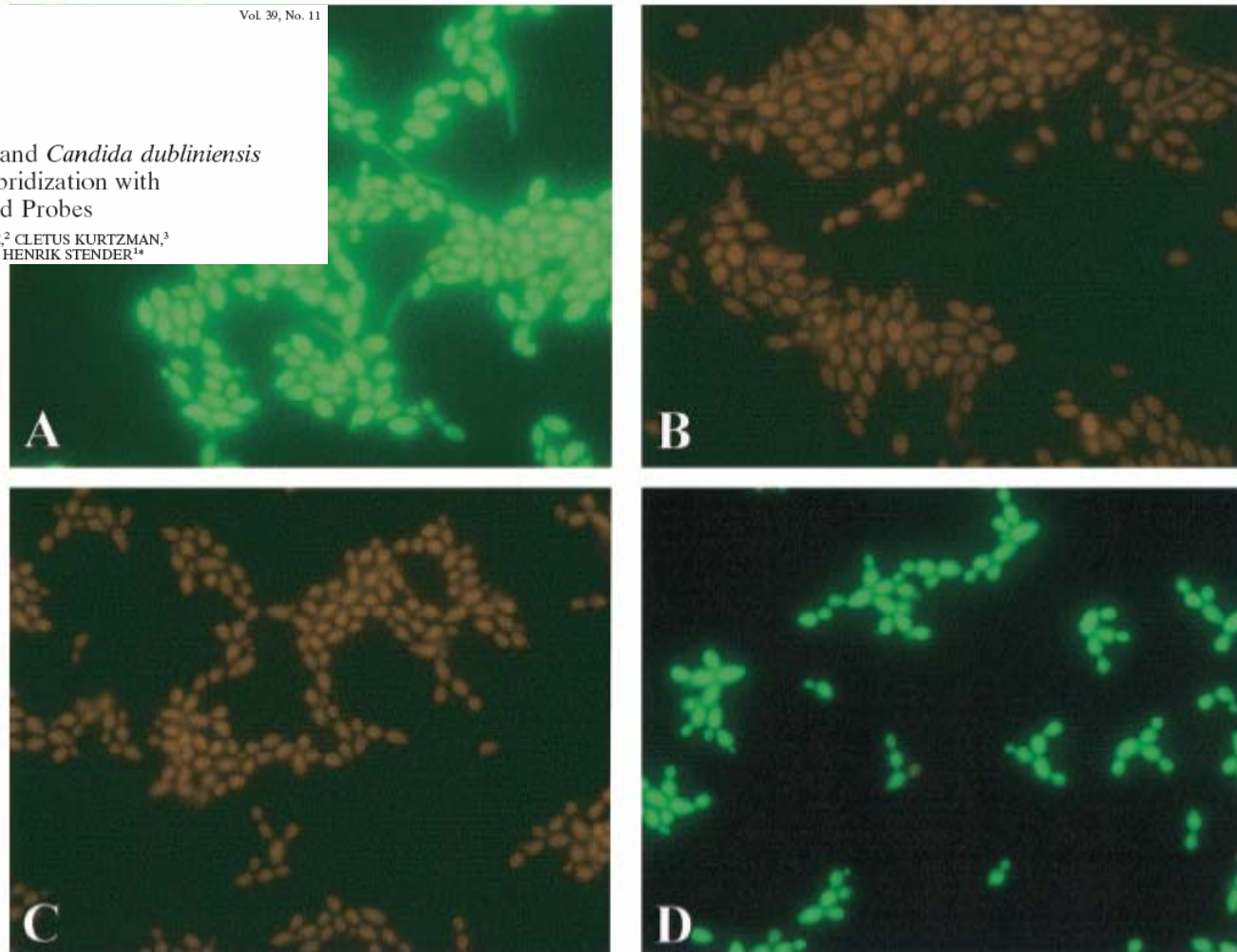


FIG. 1. Microscope images of *C. albicans* analyzed by PNA FISH using the *C. albicans* PNA probe (A) and the *C. dubliniensis* PNA probe (B) and *C. dubliniensis* analyzed by PNA FISH with the *C. albicans* PNA probe (C) and the *C. dubliniensis* PNA probe (D).



Candida-Arten im Blut

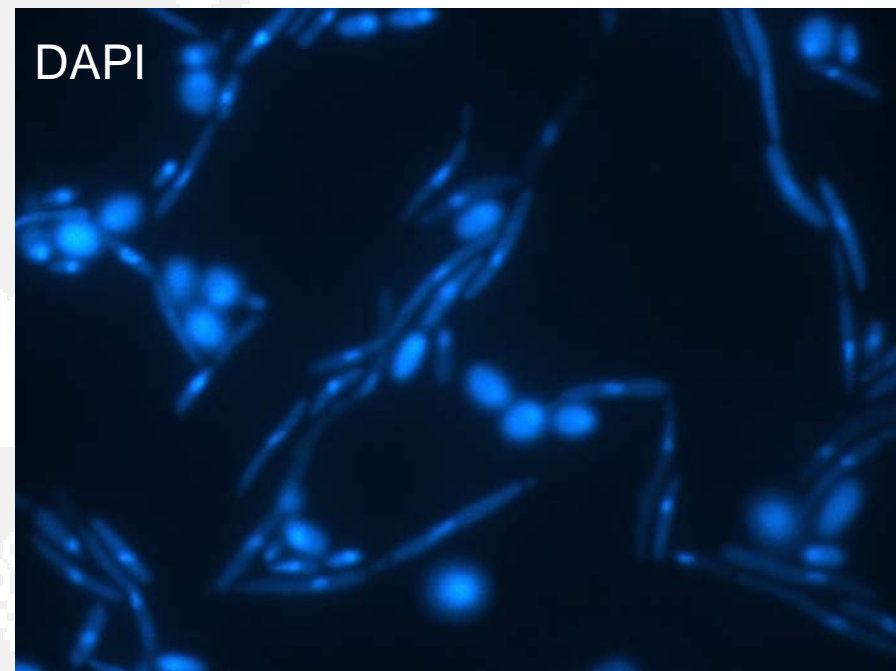
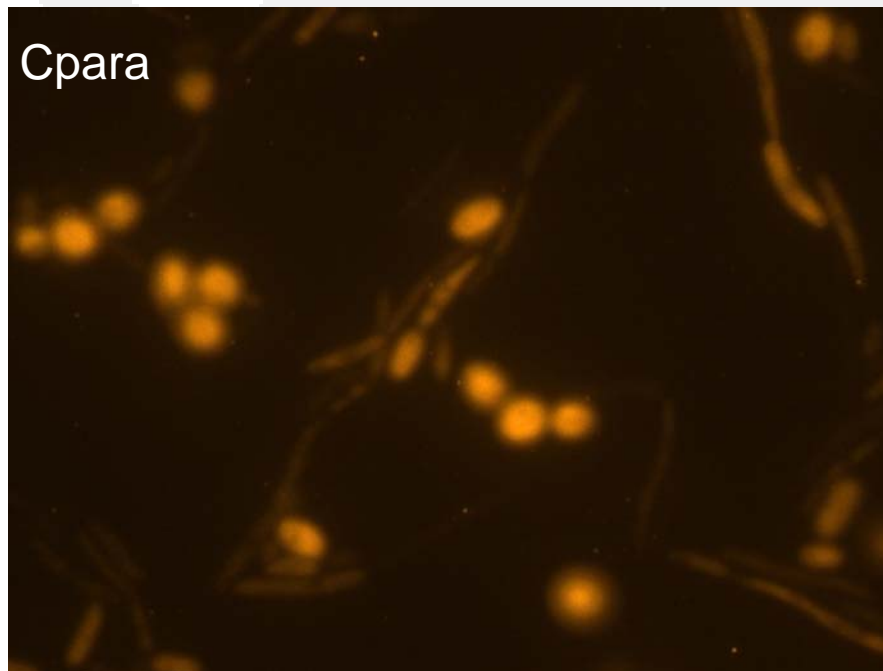
- „Home brew“



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Candida parapsilosis

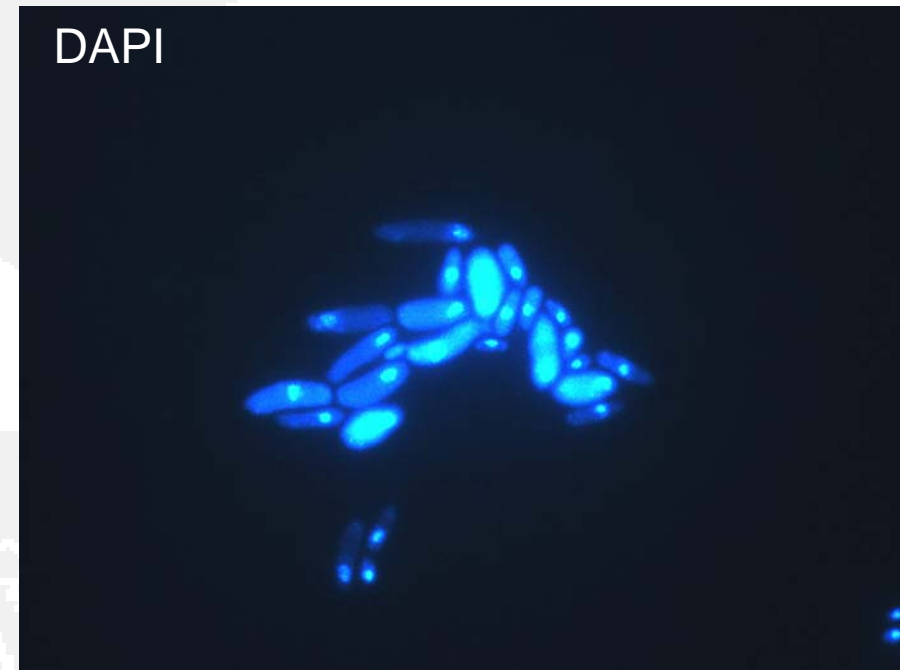


Cpara 5'-CCT GGT TCG CCA AAA AGG C-3'

18SrRNA/651



Candida krusei

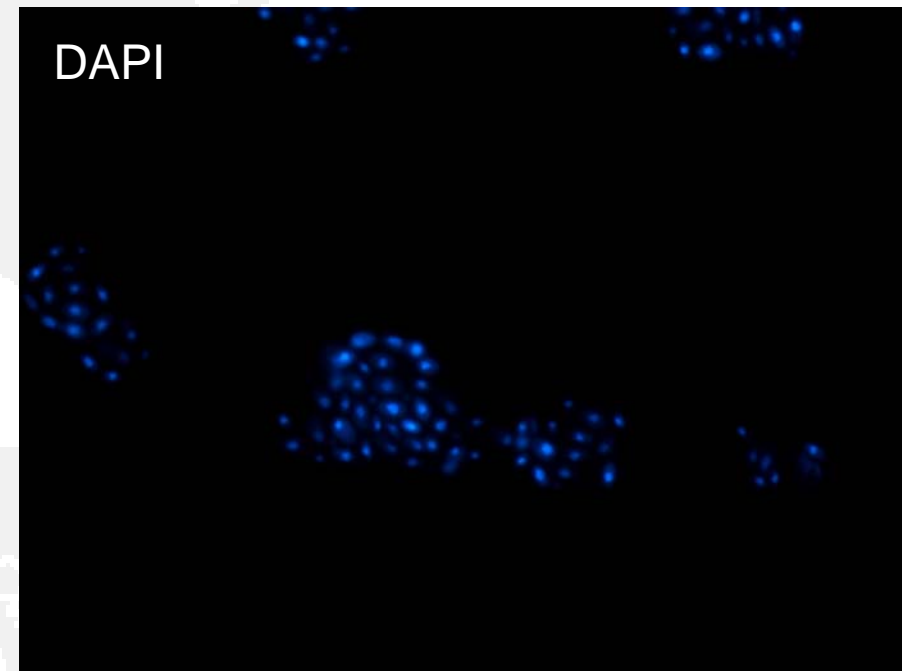


Ckrus 5'-GAT TCT CGG CCC CAT GGG-3'

18SrRNA/1433



Candida glabrata



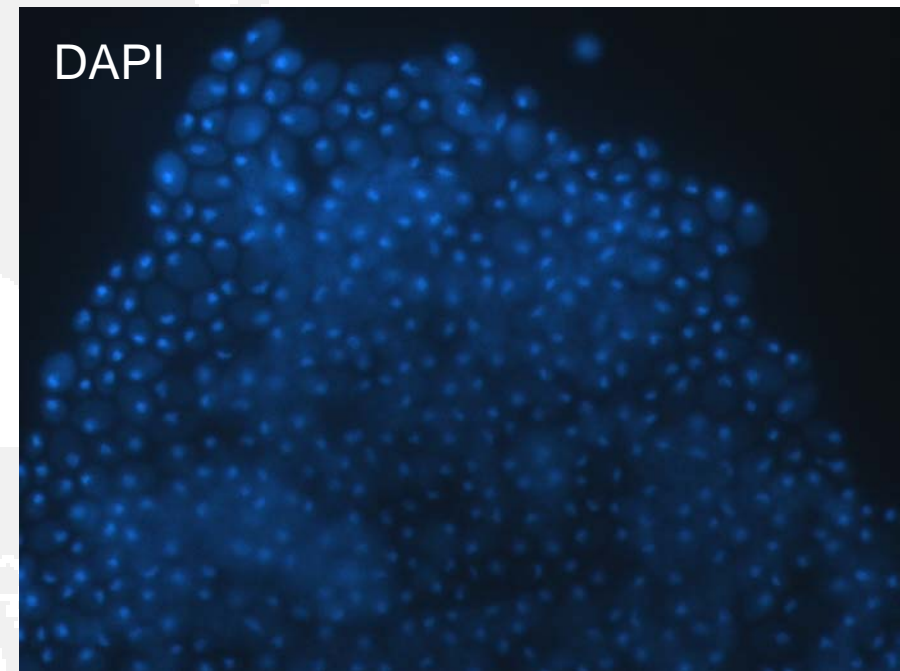
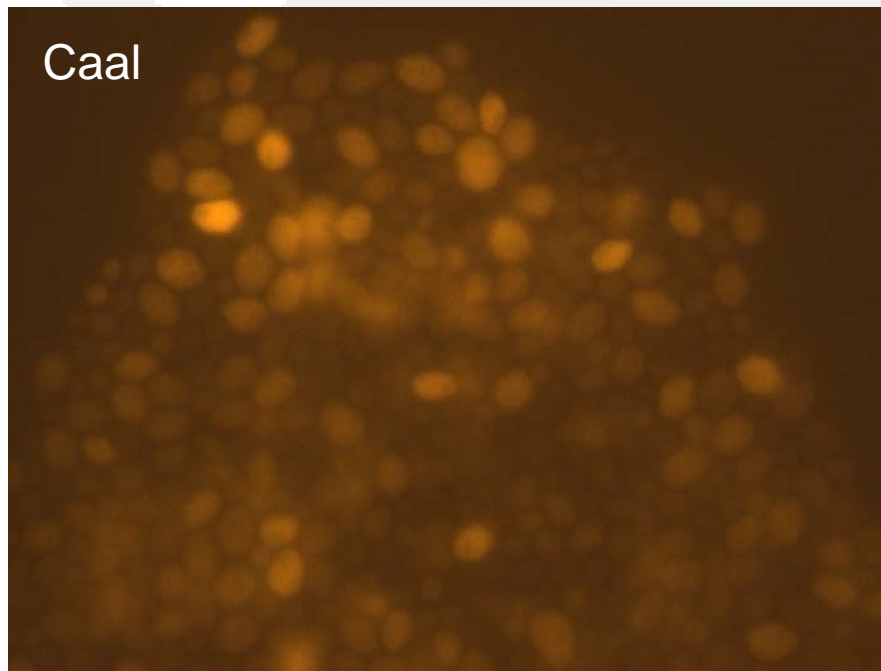
Cagl

5'-CCG CCA AGC CAC AAG GAC T-3'

18SrRNA/651



Candida albicans

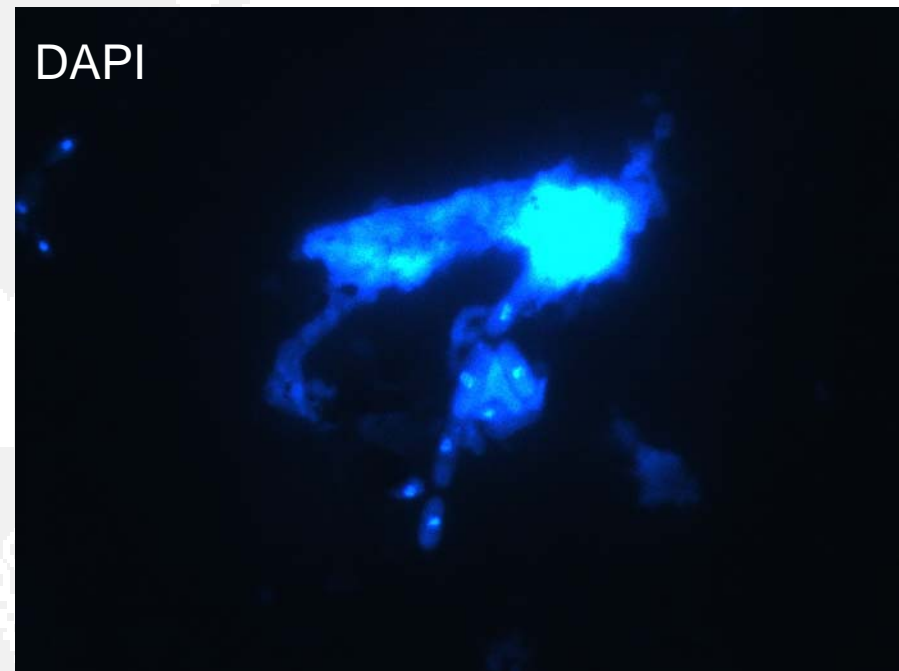


Caal 5'-GCC AAG GCT TAT ACT CGC T-3'

18SrRNA/1249



Blood: *Candida krusei*



Ckrus 5'-GAT TCT CGG CCC CAT GGG-3'

18SrRNA/1433



Candida-Arten im Blut

- Kits

Infectious Diseases / Bacteria / Viruses News

Useful Links

Video Library

AdvanDx Submits Yeast Traffic Light PNA FISH(TM) For FDA 510(k) Clearance For Detection Of Candida Species In Positive Blood Cultures

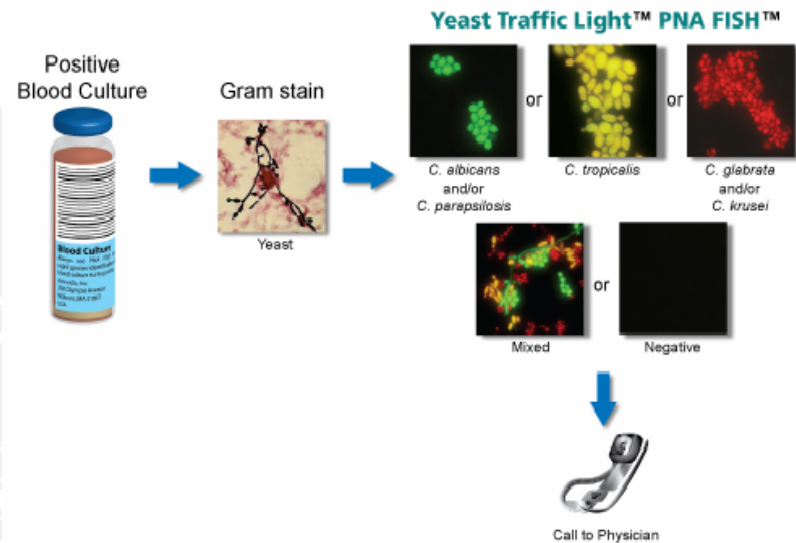
Main Category: [Infectious Diseases / Bacteria / Viruses](#)

Also Included In: [Blood / Hematology](#); [Regulatory Affairs / Drug Approvals](#); [Clinical Trials / Drug Trials](#)

Article Date: 04 Jun 2008 - 2:00 PDT

Results Reporting - Rapid Identification for 95-99% of Yeast+ Blood Cultures in Hours Instead of Days (2).

Once a blood culture turns positive, a Gram stain is performed. If the Gram stain reveals Yeast, Yeast Traffic Light™ PNA FISH is performed and results are available to be reported to the attending physician within a few hours instead of days.

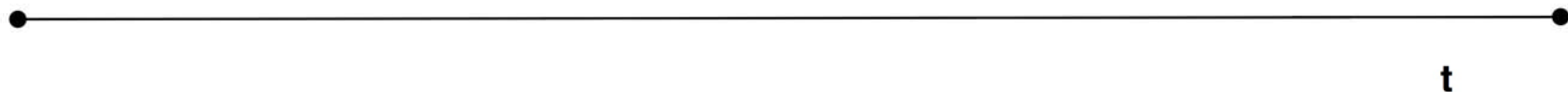
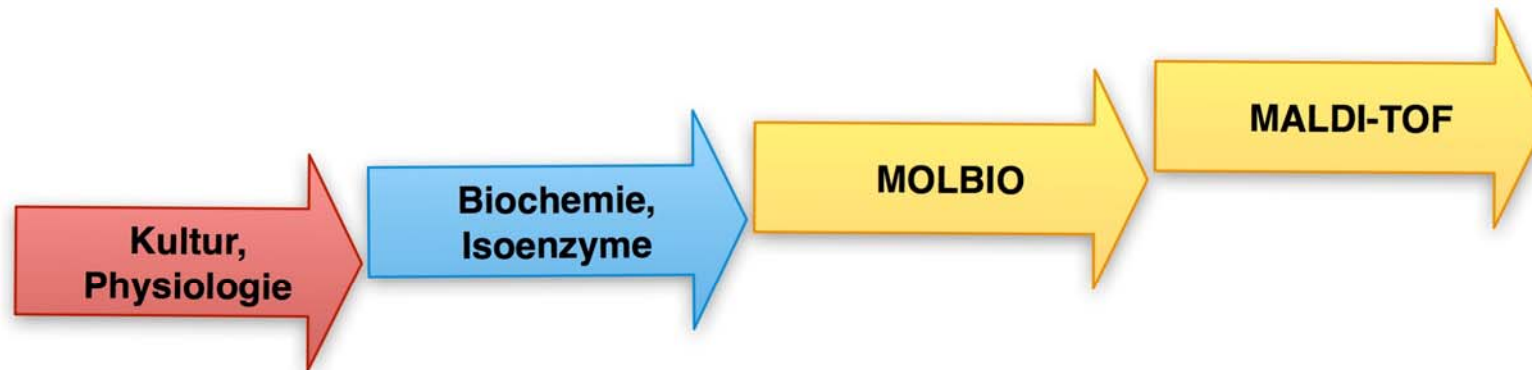


- Identifikation von Bakterien
 - 16S rDNA immer noch „Gold Standard“
 - Im Zweifelsfall: Heranziehen von weiteren Genen (z.B. *GyrB*, *rpoB*, *hsp60*) empfohlen
- Screening
 - Nutzen immer noch kontrovers; PCR-Methoden aber geeignet – cave: Kosten
- Spa-typing und andere Typisierungsmethoden für epidemiologischen Studien
- Pilze
 - ITS als geeignete Untersuchungsregion
 - Weitere Regionen: CO1, Beta-Tubulin, elongation factor 1 α (TEF), IGS
 - FISH: Differenzierung von *Candida* spp.



Diagnostik - Evolution

✚ Tod der MOLBIO?



**Fallbericht, Dr.es Stocker &
Schuler, Ospedale Civico
24.02.2010**



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K., 66-jährig

Rückkehr von Asien, Fieber

- Reise nach Asien: Thailand, Vietnam (Delta Mekong), Kambodja, Hong Kong
- 11.02. Kopfweg, Myalgie
- 13.02. Rückreise
- 13.02. in die Notfallstation eingeliefert: Puls 81/min, Blutdruck 150/68 mm Hg, Fieber(39.2°C), sat O₂ 96%, Geräusche in den Lungen

- Blutkulturen (inkl. Brucella) neg
- Malaria neg
- PCR *C. trachomatis* e *N. gonorrhoeae* (Urin) neg
- PCR H1N1 neg
- Sputum Tbc (microsc. e PCR) neg
- Sierologie
 - Hep. A, Hep. B neg
 - CMV, Toxoplasma, Trep. pallidum neg
 - Dengue neg
 - EBV neg
 - *Salmonella typhi, paratyphi, enteritidis, typhimurium*
 - *Leptospira* spp. neg
- HIV (Ag/Ac) neg

- 14.2.-16.2. Fieber 39.4 °C, Puls 76/min (“relative Bradychardie”), Blutdruck 140/70 mm Hg
- 16.2. Asthenie, Husten, Blut im Sputum
- Therapie:
 - 13.2.-16.2. Rocephin
 - 16.2. Tazobac und Klacid
 - 17.2. Tazobac und Vancocin e Klacid
- Neue Analysen:
 - Sierologie
 - *Legionella pneumophila* Immunofluor. Ig neg
 - *Coxiella burnetii* Sierologie neg
 - Viremie HIV neg
 - Pilze im Sputum neg



Diagnose nach PCR

- PCR, Sputum: Legionella
- Lungenentzündung durch Legionella-Infektion
- Behandlung mit Levofloxacin
- Besserung des Zustandes



MALDI-TOF vs PCR und Kulturen

- MALDI-TOF: schnelle Diagnose
- PCR: genaue Identifikation der Organismen
- **Kultur:** “while sequence-based identification is a powerful tool for many fungi, sequence data derived from filamentous basidiomycetes should be interpreted carefully, particularly in the context of missing or incomplete GenBank data, and, whenever possible, should be evaluated in light of compatible morphological features” (Romanelli et al., JCM 2010;48: 741)



Herzlichen Dank an...

- ICM
 - Paola Decristophoris
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 - Philippe Stocker
 - Florian Schuler