

Non-culture based diagnostics



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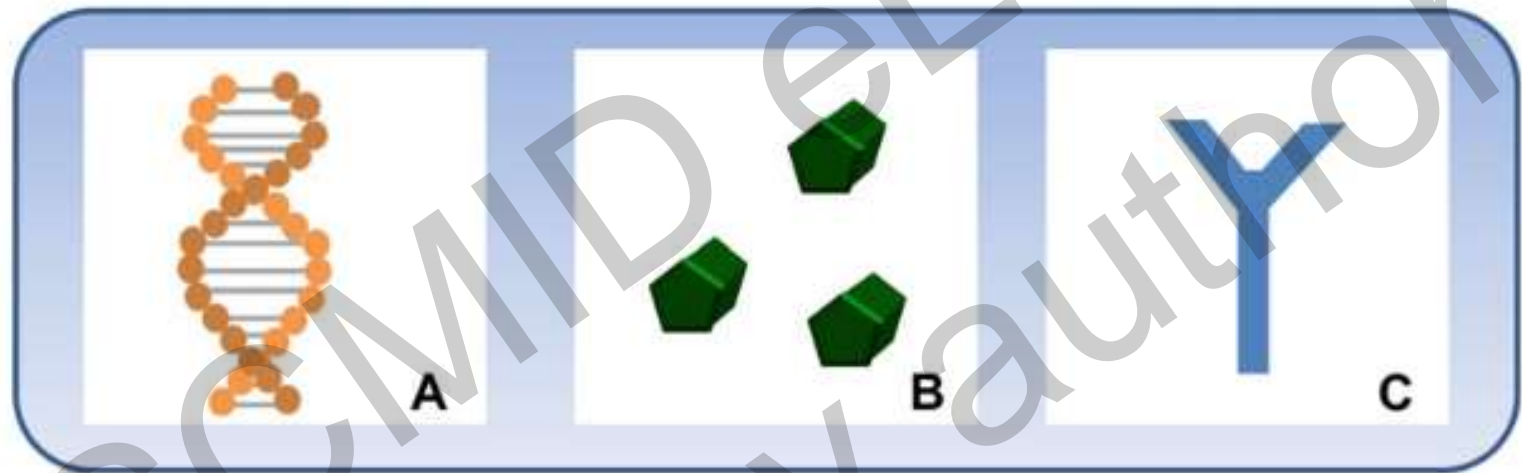


Hard facts

endogenous microflora
fungi can be both colonizers and pathogens
the finding of organisms from sputum, or GI does not necessarily indicate infection
clinical manifestations are non-specific
direct examination or cultures from sterile sites are the golden standard
conventional diagnostic tests insensitive, positive late
patients with disseminated candidiasis may have negative blood cultures
vigilance is required in the interpretation of superficial cultures, antigen tests, PCR,
presence of antibodies and/or metabolites



Target structures detected in patients samples



DNA (A), antigen (B), and antibodies (C)

Expectation & Reality



High

Sensitivity, specificity
positive predictive value
negative predictive value

....

improve patient outcome
short turnaround time
easy to handle
replace culture
stable

.....

.....

low costs
no influence of drugs

Diagnostic Tests for Invasive Candidiasis



Test and Specimen Type	β-d Glucan (panfungal marker)(blood) Fungitell, Wako
Sensitivity	65-100
Specificity	31-79
Findings from studies	<ul style="list-style-type: none"> • Performance depends on cutoff value and no. of positive samples required • Sensitivity is species-dependent: <ul style="list-style-type: none"> <i>C. krusei</i>, 100% <i>C. tropicalis</i>, 91% <i>C. albicans</i>, 83% <i>C. glabrata</i>, 81% <i>C. parapsilosis</i>, 72%
Comments	<ul style="list-style-type: none"> • Not specific for <i>Candida</i>. Positive test result requires confirmation and identification of infecting organism (<i>Aspergillus</i>, <i>Pneumocystis jiovecii</i> or <i>Candida</i>). • 2 samples a week is minimum. • Many potential sources for contamination: hemodialysis with cellulose membranes, human blood products (immunoglobulins or albumin), a moxicillin-clavulanate or piperacillin-tazobactam, severe bacterial infections, surgical sponges and gauzes containing glucan, and severe mucositis. • High negative predictive value in several studies with intermediate prevalence. However, limited sensitivity in other studies suggests that negative predictive value may be insufficient in high-risk patients.

Diagnostic Tests for Invasive Candidiasis

Test and Specimen Type	Candida mannan antigen and anti-mannan antibodies (blood or CSF)
Sensitivity	Per patient, 83 (IQR, 79-87); per sample, 62 (IQR, 55-68)
Specificity	Per patient, 86 (IQR, 82-90); per sample, 96 (IQR, 94-98)
Findings from studies	<ul style="list-style-type: none"> • Sensitivity and specificity results were given per patient and per sample • Sensitivity is species-dependent • lower for <i>C. parapsilosis</i> and <i>C. krusei</i> (40-50%) than for <i>C. albicans</i>, <i>C. glabrata</i> and <i>C. tropicalis</i> (80-100%)
Comments	<ul style="list-style-type: none"> • Combined antigen-antibody test required for maximum sensitivity. • Used to detect blood-culture negative hepatosplenic candidiasis and CNS candidiasis. • Mannan Ab testing cannot be recommended based on our study results* • <i>C. parapsilosis</i> and <i>C. guilliermondii</i> fungemias were not detected by the Platelia Candida Ag Plus assay** • Limited experience

CFS, cerebrospinal fluid; CNS, central nervous system; IQR, interquartile range;



Kullberg et al. N Engl J Med 2015;373:1445

*Hönigl et al., Mycoses 2016, 59(6):374-8

**Lass-Flörl. Methods Mol Biol. 2017;1508:3-15

Diagnostic tests for invasive Candidiasis

Test and Specimen Type	PCR assay (blood), noncommercial
Sensitivity	82-98%
Specificity	87-98%
Findings from studies	<ul style="list-style-type: none"> • Patients had candidemia or invasive candidiasis; results based on meta-analysis of range of inhouse multiplex PCR assays
Comments	<ul style="list-style-type: none"> • PCR formats specific for detection of Candida preferred since they are less prone to contamination by airborne fungi and fungal DNA. • In general, sensitivities are similar to those of culture results for candidemia and better for deep-seated candidiasis, with shorter turnaround time. • Lack of multicenter validation. • For deep-seated candidiasis, sensitivity and specificity higher than with β-d-glucan.

PCR, polymerase chain reaction

Kullberg et al. N Engl J Med 2015;373:1445



Examples on commercially available DNA-detecting methods for clinical specimens

Assay	Methods	Fungi	Sensitivity (%)	Specificity (%)	Detection limit	Processing time	Specimens
ePlex-BCID-FP GenMark DX „Bedside test“	Ready to use (DNA hybridization and electrochemical detection)	16 fungal targets: <i>C. albicans</i> <i>C. dubliniensis</i> <i>C. famata</i> <i>C. glabrata</i> <i>C. guilliermondii</i> <i>C. kefyr</i> <i>C. krusei</i> <i>C. Lusitaniae</i> <i>C. Parapsilosis</i> <i>C. tropicalis</i>	-	-	-	1.5 h	Positive blood cultures
FilmArray® BCID Panel Biomerieux	Ready to use Multiplex PCR	<i>C. albicans</i> <i>C. glabrata</i> <i>C. krusei</i> <i>C. parapsilosis</i> <i>C. tropicalis</i>	100	99.8-100	-	1 h	Positive blood cultures
T2 Candida Panel T2 Biosystems	Ready to use (magnetic resonance assay)	<i>C. albicans</i> <i>C. Tropicalis</i> <i>C. parapsilosis</i> <i>C. krusei</i> <i>C. glabrata</i>	91.1	99.4	1 cfu/mL	4.5 h	Whole blood
IRIDICA BAC BSI Abbott Diagnostics	PCR & mass spectrometry	Panmicrobial	81	84	8 cfu/mL	6 h	Whole blood, sterile fluids, tissue, BAL, endotracheal aspirate

Tests vary in the target, sen., spec., turnaround time and specimen application!

Diagnostic tests for invasive Candidiasis

Test and Specimen Type	PCR assay (blood), SeptiFast
Sensitivity	48-72%
Specificity	99%
Findings from studies	<ul style="list-style-type: none"> • Results based on meta-analysis
Comments	<ul style="list-style-type: none"> • Detects <i>C. albicans</i>, <i>C. glabrata</i>, <i>C. krusei</i>, <i>C. parapsilosis</i>, <i>C. tropicalis</i>, and <i>Aspergillus fumigatus</i> • Labor-intensive • Risk of false positive results for <i>Aspergillus</i>
Test and Specimen Type	PCR assay (blood), T2Candida Panel
Sensitivity	91%
Specificity	94%
Findings from studies	<ul style="list-style-type: none"> • Multicenter study among 1501 patients (6 of 1501 candidemic) and additional 250 spiked samples
Comments	<ul style="list-style-type: none"> • Detects <i>C. albicans</i>, <i>C. glabrata</i>, <i>C. krusei</i>, <i>C. parapsilosis</i>, <i>C. tropicalis</i>. • Appears promising but validation in higher-risk populations needed.

PCR, polymerase chain reaction

*A spiked sample is a negative sample to which Candida has been added

Performances of BDG (cutoff 80,100 and 200 pg/mL), CAGTA, MANNAN biomarkers and C-PCR used alone for IC diagnosis

Invasive candidiasis	Sensitivity % (95 % CI)	Specificity % (95 % CI)	NPV % (95 % CI)	PPV % (95 % CI)
BDG ≥ 80 pg/mL	76.7 (57.7–90.1)	57.2 (49.9–64.3)	94.1 (89.1–96.8)	21.7 (17.7–26.4)
BDG ≥ 100 pg/mL	70.0 (50.6–85.3)	61.5 (54.3–68.4)	93.0 (88.4–95.9)	21.9 (17.3–27.3)
BDG ≥ 200 pg/mL	60.0 (40.6–77.3)	79.4 (73.1–84.8)	92.9 (89.4–95.4)	30.5 (22.7–39.6)
CAGTA positive	53.3 (34.3–71.7)	64.3 (57.2–71.0)	90.1 (86.0–93.2)	18.4 (13.3–24.8)
Mannan-Ag positive	43.3 (25.5–62.6)	67.3 (60.3–73.8)	88.7 (85.0–91.6)	16.7 (11.3–24.0)
Mannan-Ab positive	25.8 (11.9–44.6)	89.0 (83.8–93.0)	88.6 (86.2–90.6)	26.7 (15.1–42.6)
C-PCR positive	84.0 (63.9–95.5)	32.9 (23.1–44.0)	87.5 (73.1–94.8)	26.9 (22.7–31.6)

Values are frequencies and percentages. Biomarker positives: two determinations consecutives positives (cutoff). C-PCR positive: one determination positive

BDG (1-3)- β -glucan, *CAGTA* *Candida albicans* germ tube antibody, *mannan-Ag* mannan antigen; *mannan-Ab* mannan antibody, *C-PCR* PCR-based *Candida* detection

***Candida albicans* germ-tube antibody: Evaluation of a new automatic assay for diagnosing invasive candidiasis in ICU patients**

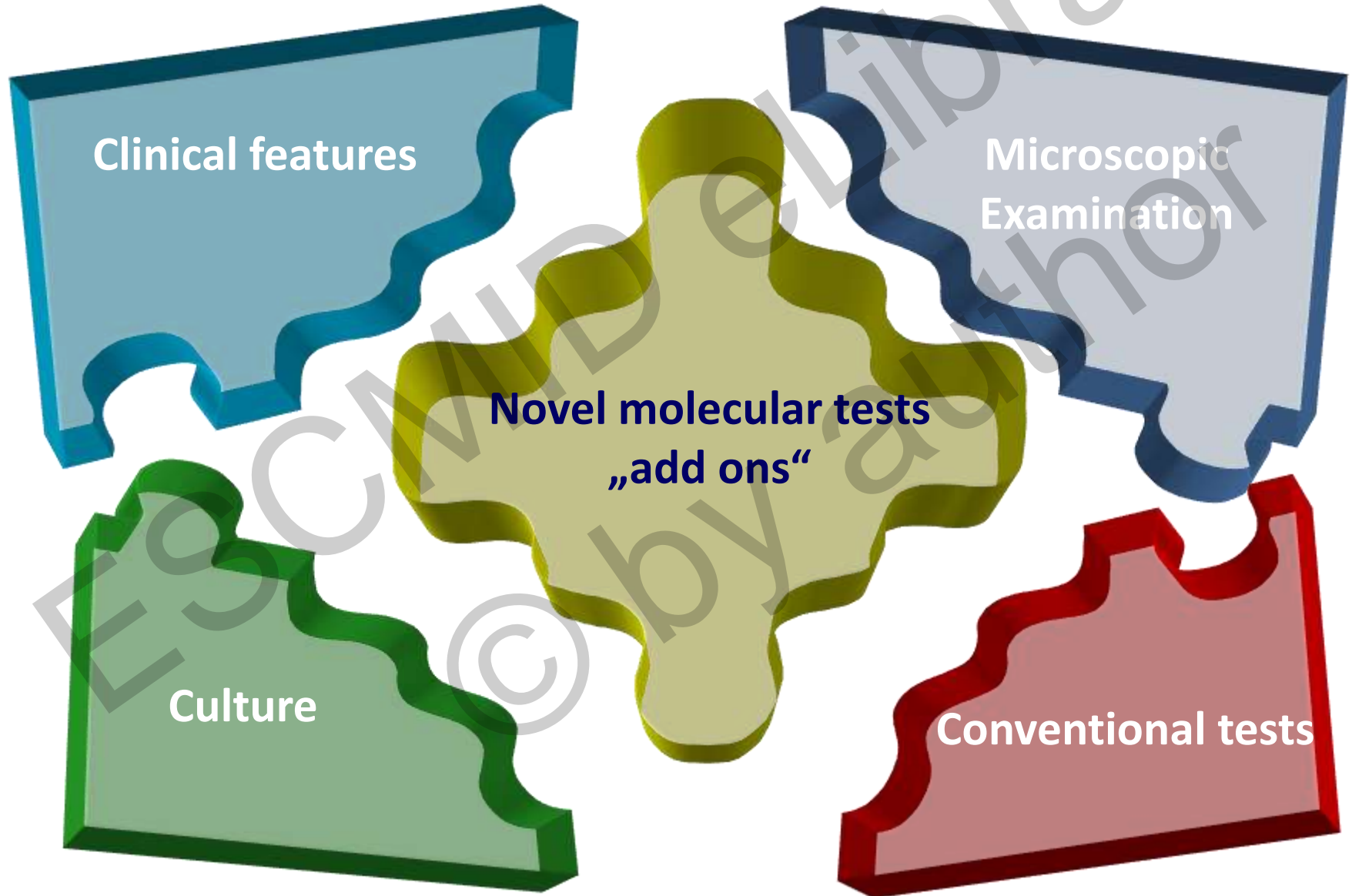
- Testing for *Candida albicans* germ-tube antibody IFA IgG assay (CAGTA) is used to detect invasive candidiasis.
- The CAGTA assay was adapted in an automatic monotest system (invasive candidiasis [CAGTA] VirClia[®] IgG monotest (VirClia[®]).
- CAGTA assay was compared with the monotest automatic VirClia[®] assay
- A prospective study with 361 samples from 179 non-neutropenic critically ill patients was conducted. 21 patients with candidemia.
- Overall agreement between the two assays (CAGTA and VirCLIA) was 85,3%. Assays were compared with gold-standard method to determine the sensitivity, specificity as well as positive and negative predictive values. In patients with candidemia, values for CAGTA and VirCLIA assays were 76.2 versus 85.7%, 80.3 versus 75.8%, 55.2 versus 52.9%, and 91.4 versus 94.3%, respectively.
- According to theses results, the automated VirClia[®] assay was a reliable, rapid, and very easy to perform technique as tool for the diagnosis of invasive candidiasis.

An evaluation of the performance of the Dynamiker[®] Fungus (1-3)- β -D-Glucan assay to assist in the diagnosis of invasive aspergillosis, invasive candidiasis and Pneumocystis pneumonia

- 163 serum samples from 121 patients tested
 - 28 proven IC cases and 64 control patients
- D-BDG showed fair and good agreement with the Fungitell[®]
- D-BDG provides a useful adjunct test to aid the diagnosis of IFD, with technical flexibility that will assist laboratories processing low sample numbers.



The future: „Puzzle diagnosis“





YEAST INFECTIONS

Self-Test*

for a Candida Infection

- | | |
|---|--|
| 1. Do you feel tired most of the time or have muscle aches with normal activity? | 4. Do you suffer from mood swings, irritability, anxiety or depression? |
| 2. Do you suffer from intestinal discomfort—bloating, constipation and/or diarrhea? | 5. Are you ever dizzy, light-headed or have trouble concentrating or thinking clearly? |
| 3. Do you crave sugar, breads, beer or other alcoholic beverages? | 6. Have you ever used antibiotics, birth control pills or steroid drugs? |

Three or more "yes" answers indicate a high to very high probability that you have a yeast infection. Control helps combat that infection.



Thank you for your attention!