



## Is there a role for antibody testing in the diagnosis of invasive candidiasis?

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### Summary

During the last decades, the use of antibody tests for the diagnosis of invasive mycoses has declined as a consequence of the general belief that they are insensitive and non-specific. However, there is a clear evidence that antibodies can be detected in highly immunodeficient patients (such as bone marrow transplant recipients), and that those antibodies are useful for the diagnosis. Antibody tests are currently in use as diagnostic tools for some primary mycoses, such as the endemic mycoses, aspergilloma, allergic broncho-pulmonary aspergillosis and sporotrichosis. For invasive candidiasis, diagnostic methods must differentiate *Candida* colonization of mucous membranes or superficial infection from tissue invasion by this microorganism. Substantial progress has been made in diagnosis of invasive candidiasis with the development of a variety of methods for the detection of antibodies and antigens. However, no single test has found widespread clinical use and there is a consensus that diagnosis based on a single specimen lacks sensitivity. It is necessary to test sequential samples taken while the patient is at greatest risk for developing invasive candidiasis to optimize the diagnosis. Results obtained from a panel of diagnostic tests in association with clinical aspects will likely be the most useful strategy for early diagnosis and therapy.

### Key words

Candidiasis, Diagnosis, Antibody, Anti-germ tube, Anti-mannan, Detection

## ¿Es útil la detección de anticuerpos en el diagnóstico de la candidiasis invasiva?

### Resumen

En las últimas décadas, el empleo de pruebas serológicas para la detección de anticuerpos en el diagnóstico de las micosis invasoras ha sufrido un continuo declive debido a la creencia generalizada de que son pruebas poco sensibles e inespecíficas. Sin embargo, hay evidencias claras de que se detectan anticuerpos en pacientes inmunosuprimidos (como en los receptores de trasplante de médula ósea) y que esta detección tiene utilidad diagnóstica. Las pruebas serológicas de detección de anticuerpos se emplean habitualmente como herramientas diagnósticas en algunas infecciones fúngicas, como las micosis endémicas, el aspergiloma, la aspergilosis bronco-pulmonar alérgica y la esporotricosis. En las candidiasis invasoras, los métodos diagnósticos deben ser capaces de diferenciar la colonización de las mucosas por *Candida* o las candidiasis superficiales, de la invasión de los tejidos por este microorganismo. Se han conseguido avances importantes con el desarrollo de diversos métodos de detección de anticuerpos. Sin embargo, ninguna prueba ha conseguido un uso clínico amplio y hay un consenso sobre la inutilidad diagnóstica de estudiar una sola muestra y que es necesario evaluar muestras séricas seriadas tomadas cuando el paciente está en mayor riesgo de desarrollar una candidiasis invasora para mejorar el diagnóstico. Los resultados obtenidos empleando una batería de pruebas diagnósticas asociados a los aspectos clínicos del paciente son probablemente la estrategia más útil para realizar un diagnóstico y un tratamiento tempranos.

### Palabras clave

Candidiasis, Diagnóstico, Anticuerpos, Anti-micelio, Anti-mannano, Detección

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Serological methods used in the diagnosis of invasive candidiasis rely on the detection of antibodies against *Candida*, detection of *Candida* antigens and detection of non-antigenic *Candida* components, like D-arabinitol, mannose, 1→3-β-D-glucan or nucleic acids. In this paper, we review the current status on diagnosis of invasive candidiasis by detecting antibodies against different antigens of *Candida*.

Diagnostic methods must differentiate *Candida* colonization of mucous membranes or superficial infection from tissue invasion and candidemia requiring antifungal therapy [36]. In performing this task, two main limitations are evident: the specificity of tests may be low because antibody titers can be high in colonized patients [9], and tests may present a low sensitivity because the antibody response may be delayed, reduced or absent [7]. However, it is possible to overcome these limitations because the specificity of the tests can be improved by selecting the appropriate antigens (purified molecules, recombinant antigens, etc.) and sensitivity of the tests can be increased by using sensitive and standardized commercial techniques, such as the ELISA. A useful test must combine improvements in both sensitivity and specificity, since nothing is gained from a more sensitive test unless a more specific antigen is also used [8]. Although, there is a general belief that antibody tests are both insensitive and non-specific [9,20,34], there is important recent evidence suggesting that detection of antibodies in highly immunocompromised patients, such as neutropenic bone marrow transplant recipients or liver transplant recipients, is possible and useful for the diagnosis of invasive candidiasis [18,24,32,54,56]. Since the main limitations of antibody detection (low sensitivity and specificity) are especially manifested when only a single serum sample is studied, detection of antibodies by commercially available test kits with multiple sequential sera from the patient at risk for developing invasive candidiasis should be performed. This will allow a kinetic study of the antibody response in the patient to be performed, which will provide a more reliable reflection of the development of the infection [11,19,21,22,25,26,27,51].

Mannan is an abundant antigen located on the *Candida* cell wall surface [37]. This antigen is highly immunogenic for humans, who usually respond with a rise in the anti-mannan antibody titers when *Candida* enters the bloodstream. Anti-mannan antibodies have been detected in many studies [36]. Detailed information about the kinetics of anti-mannan antibodies during invasive candidiasis was obtained in the studies performed by Jones and associates [16,21,22]. A sensitivity of 53% and a specificity of 94% in the diagnosis of invasive candidiasis in immunocompetent patients, have been reported for anti-mannan antibody detection by using a commercially available ELISA (Platelia *Candida* Ab, Bio-Rad, France) in sequential serum samples [51]. Other studies have shown lower diagnostic values (sensitivity, 59% to 90.9%; specificity, 18.4% to 63.1%) using this and other commercial tests, such as Candidquant (Biomerica, USA) and *Candida albicans* ELISA (Virotech GmbH, Germany) [33].

A mannoprotein of 230-250 kDa located on the germ tube cell wall surface is recognized by sera from patients with invasive candidiasis. Our group [36,38,41,42] has developed an indirect immunofluorescence assay to detect antibodies (CAGTA) against this antigen present in *C. albicans* germ tubes that has been useful in the diagnosis of invasive candidiasis in different groups of patients, including intravenous heroin users [36,38,40,44], bone marrow transplant recipients [15], patients with hematological disorders and intensive care patients [15,19,30,46].

The test has shown an overall sensitivity of 77-89% and a specificity of 91-100% (Table). These results are in contrast to those obtained when antibodies to *C. albicans* blastoconidia were detected, since detection of antibodies to *C. albicans* blastoconidia, which are mainly directed against mannans, is more sensitive than detection of CAGTA, but less specific [38,41,42,55,56]. Sera sequentially drawn from patients at risk of developing invasive candidiasis showed CAGTA before the microbiological diagnosis was made, especially in patients with tissue proven invasive candidiasis and detection of CAGTA seemed to complement and even anticipate blood culture [15]. Detection of CAGTA in patients with invasive infections caused by *Candida* species other than *C. albicans* (*Candida tropicalis*, *Candida parapsilosis*, *Candida glabrata*, *Candida dubliniensis*, *Candida guilliermondii* and *Candida krusei*) may also be positive, although titers are lower than in candidiasis by *C. albicans* [3,15,19,30,38,41,43,46,48]. In addition, detection of CAGTA may be useful for the therapeutic monitoring of patients with invasive candidiasis, since the administration of antifungal therapy usually results in decreasing titers of CAGTA [19,30,44,48].

*Candida albicans* IFA IgG (Viracell Laboratories, Spain) has been recently commercialized for CAGTA detection. This test has been compared in a retrospective study [30] to our standard test using 172 sera from 51 hematological and intensive care patients (123 sera from 32 patients with invasive candidiasis and 49 sera from 19 patients without evidence of infection by *Candida*). *Candida albicans* IFA IgG test showed a sensitivity of 84.4% and a specificity of 94.7%, while the standard test showed a sensitivity of 78.1% and a specificity of 100%. Results with both techniques presented a high correlation ( $R^2 = 0.9512$  by patients,  $R^2 = 0.8986$  by sera). The commercially available *Candida albicans* IFA IgG test was similar to the standard test and provided faster and easier diagnosis of invasive candidiasis in the clinical microbiology laboratory [30].

CAGTA present in patients with invasive candidiasis are likely to be directed against both *C. albicans* type I and II antigens. Pontón et al. [38] described six different types of antigens in the *C. albicans* cell wall. Type I antigens are truly germ tube-specific and are expressed on the germ tube cell wall surface only. Type II antigens are expressed on both the germ tube cell surface and within the blastoconidium cell wall. These antigens would appear as germ tube-specific if tested by indirect immunofluorescence, but they can be extracted from the blastoconidium cell wall with treatments which remove the outermost layers. Presence of type II antigens within the blastoconidium cell wall would explain the induction of antibodies to germ tubes in rabbits immunized with dithiothreitol extracts from blastoconidia, heat killed blastoconidia and formalin killed blastoconidia [45], or in both patients [41] and rabbits [3] infected with *Candida* species different from *C. albicans* which are unable to produce germ tubes in serum. Type III antigens are expressed on both the blastoconidium and germ tube surface. Type IV antigens are expressed within the cell wall of both blastoconidia and germ tubes. Type V antigens are expressed on the blastoconidium cell surface and within the germ tube cell wall and Type VI antigens are expressed on the blastoconidium surface only. Since the germ tube cell wall also contains mannan, to detect CAGTA the sera have to be adsorbed, a process that is time consuming and requires large amounts of heat-killed blastoconidia. A test based on the detection of antibodies against type I antigens may not require the adsorption of the sera and would therefore

facilitate serodiagnosis of invasive candidiasis. In this regard, by using an ELISA and purified *C. albicans* Type I and II antigens, Bikandi et al. [4] detected antibodies in sera from patients with invasive candidiasis with a sensitivity of 78% and a specificity of 68% without removing the anti-mannan antibodies from the sera. In a similar study, Berdin et al. [2] developed an ELISA to detect IgG antibodies against a *C. albicans* Type I antigen in patients with invasive candidiasis and reported a sensitivity of 78% and a specificity of 82%.

*Candida* antigens with enzymatic activity (enolase, aspartyl proteinase and metalloproteinase) have also been used as targets for antibody detection with controversial results, and commercial kits are not available at present [10,28,29,53]. Antibody response against enolase has shown a sensitivity of 50 to 92% and a specificity of 86-95% for the diagnosis of invasive candidiasis in immunocompetent patients and a sensitivity of 53% and a specificity of 78% in immunodeficient patients [10,28,29]. Detection of antibodies against a *Candida albicans* secreted aspartyl proteinase in patients with invasive candidiasis had a sensitivity of 69.7% and a specificity of 76% [31,47]. Antibodies against a *C. albicans* metalloproteinase by ELISA showed a sensitivity of 83% and a specificity of 97% [12].

Although in most studies only anti-*Candida* IgG antibodies were detected, other immunoglobulin classes have been also investigated in some studies (Table). Aubert et al. [1] used an immunocapture technique to detect IgM, IgA and IgE anti-*Candida* antibodies against somatic antigens in immunocompetent patients and found that IgA was a particularly valuable marker of invasive candidiasis. Detection of IgA CAGTA showed a higher sensitivity than IgG or IgM detection in the diagnosis of invasive candidiasis [40]. Gutierrez et al. [17] detected IgM antibodies to *C. albicans* whole cells by indirect immunofluorescence in patients with first time candidemia and reported a 100% sensitivity and specificity. Kostiala et al. [25] detected rises in titers of IgG and IgA antibodies by ELISA in sera sequentially drawn from patients with candidemia.

Combinations of tests for detection of antibodies and antigens [14,23,49-52], and antibodies, antigens and non antigenic components [5,6,13,35] may be useful to overcome the deficiencies of individual tests. Different combinations of tests used in individual studies make comparisons between studies difficult, but combinations of tests which detect antibodies and antigens have been found to improve diagnosis of invasive candidiasis [14,49-52]. The need for a combination of tests is particularly evident when a specific antigen and antibodies to this antigen are detected in the same patient, since the antibodies may facilitate the clearance of the antigen. Detection of mannan

and anti-mannan antibodies have been shown to be complementary in patients with invasive candidiasis [49-51], since patients with antigenemia did not have significant levels of anti-mannan antibodies or vice versa. Detection of anti-mannan antibodies was inversely correlated to the antigenemia. For combined results of both tests, a sensitivity of 80% to 95.4% and a specificity of 52.6% to 93% have been reported [49-51,57]. Platenkamp et al. [35] reported a sensitivity of 77% and a specificity of 100% when a combination of antibody, antigen and D-arabinitol detection was used to differentiate invasive candidiasis from *Candida* colonization in immunocompromised patients. However, Bougnoux et al. [6] did not find the combination of antibody, antigen and D-arabinitol assays useful to differentiate disseminated from peripheral candidiasis.

## Conclusions

Substantial progress has been made in diagnosis of invasive candidiasis with the development of a variety of methods for the detection of antibodies and antigens. However, no single test has found widespread clinical use due to the difficulties in obtaining consistently reliable serological diagnosis in all patients with invasive candidiasis and due to the limited number of commercial assays available. There is a consensus in the literature that diagnosis based on a single specimen lacks sensitivity. Therefore, it is necessary to test sequential samples taken while the patient is at greatest risk for developing invasive candidiasis to optimize the diagnosis. Testing of serum samples should begin at patient admission to obtain baseline data and consideration should be given to the fact that different assays may be needed for the diagnosis of invasive candidiasis in immunocompetent and immunocompromised patients. Furthermore, patients should be screened taking into account the different expected kinetics of each assay (v.g.: weekly for antibody detection). Results obtained from a panel of diagnostic tests in association with blood culture findings and clinical aspects of the patient, will likely be the most useful strategy for early diagnosis of patients with invasive candidiasis and monitoring of therapeutic response.

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**Table.** Recent studies on antibody detection for the diagnosis of invasive candidiasis.

Year	Method	Antigen	Sensitivity (%)	Specificity (%)	Reference
1990	IFA	BCW (SER A)	45.5	60.9	[6]
1990	IFA	BCW (SER A)	50	62.5	[6]
1990	IFA	MCW	77.8	96.8	[41]
1992	ELISA (IgM)	BC	35.7	90.1	* [52]
1993	IFA (IgM)	BC + MAN	100	100	* [17]
1993	IFA (IgG)	BC + MAN	80	81.4	* [17]
1993	ELISA	BC	46.4	100	[32]
1993	ELISA	MCW	82.9	98.6	[32]
1993	IFA	MCW	100	97.7	[56]
1994	IFA	MCW	84.6	87.9	* [38]
1994	ELISA	ENO	50	100	* [10]
1994	ELISA	ENO	53	95	[10]
1995	IFA	BCW	57	85	[46]
1995	IFA	MCW	82	94	[46]
1995	ELISA	ENO	73	95	* [2]
1996	CIE	BC + MAN	64	89	[11]
1996	CoCIE	BCW + S	58.9	96.4	* [1]
1996	IFA	BCW + S	48.2	96.4	* [1]
1996	HA	MAN	64	86	[11]
1997	IFA	MCW	87.5	95.2	[15]
1997	IFA (IgA)	MCW	40.9	100	* [55]
1997	IFA (IgG)	MCW	40.9	100	* [55]
1997	IFA (IgM)	MCW	9.1	100	* [55]
1998	ELISA	52 kDA METAL	83	97	* [12]
1999	ELISA	MAN	53	94	* [51]
2002	ELISA	MAN	59	63.1	[33]
2002	ELISA (IgG)	MAN	77.2	36.8	[33]
2002	ELISA (IgG)	MAN	90.9	18.4	[33]
2002	ELISA	S	59	63.1	[33]
2002	CIE	S	59	55.2	[33]
2002	IFA	BC	54.5	63.1	[33]
2004	IFA	MCW	84.4	94.7	[30]

\*Not including neutropenic patients with superficial candidiasis among the controls. **Methods:** CIE (Counterimmunoelectrophoresis), CoCIE (CoCounterimmunoelectrophoresis), ELISA (Enzyme-linked immunosorbent assay), HA (Hemagglutination), and IFA (Immunofluorescence Assay). **Antigens:** METAL (Metalloproteinase), BC (Blastospore Cytoplasm), MAN (Mannan), BCW (Blastospore Cell Wall), SER A (Serotype A), ENO (Enolase), MCW (Mycelium Cell Wall), and S (Somatic).

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