Review

# The application of monoclonal antibodies to the diagnosis of disseminated mycoses

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*Summary*

Monoclonal antibodies (MoAbs) have had a major impact on many areas of biomedical research and almost since their advent have been used in the characterisation and identification of diagnostically important antigens of fungal pathogens. Their main significance lies in three, often inter-related areas: a) the definition and characterisation of antigens for use in detection of antibody responses, b) their direct use in the detection of diagnostically useful antigen in body fluids c) their application in immunohistochemical diagnosis. The degree to which MoAbs have been applied varies between fungal pathogens, and they have now been used, for example, in the serodiagnosis of *Aspergillus* sp., *Cryptococcus neoformans, Histoplasma capsulatum* and *Paracoccidioides brasiliensis*. Their use in producing diagnostic tests for other fungi such as *Sporothrix schenckii* and *Penicillium marneffei* has been more restricted but considerable potential exists for further development.

*Key words*

Diagnosis, monoclonal antibodies, fungal pathogens

## La aplicación de anticuerpos monoclonales al diagnóstico de las micosis diseminadas

*Resumen*

Los anticuerpos monoclonales (AcM) han tenido un gran impacto en varias áreas de la investigación biomédica y prácticamente desde su aparición han sido utilizados en la caracterización e identificación de antígenos con interés diagnóstico de patógenos fúngicos. Su principal interés reside en tres aspectos a menudo relacionados entre sí: a) la definición y caracterización de antígenos útiles para la detección de la respuesta de anticuerpos, b) su utilización directa en la detección en fluídos corporales de antígenos con significado diagnóstico, c) su aplicación en el diagnóstico inmunohistoquímico. El alcance de la aplicación de los AcM varía entre los diferentes patógenos fúngicos y se han utilizado, por ejemplo, en el serodiagnóstico de *Aspergillus* sp., *Cryptococcus neoformans, Histoplasma capsulatum* y *Paracoccidioides brasiliensis*. Su utilización para el desarrollo de pruebas diagnósticas para otros hongos como *Sporothrix schenckii* y *Penicillium marneffei* ha sido más limitada pero existe un considerable potencial para su desarrollo posterior.

Diagnóstico, anticuerpos monoclonales, patógenos fúngicos

Generally speaking the gold standards for the diagnosis of disseminated fungal infections are cultural isolation and positive histochemical identification. However serological detection of fungal infections is often of great importance, both at the point of diagnosis and during the monitoring of patients response to therapy. This is of particular importance in situations were cultural procedures fail, or where histochemical identification proves to have low sensitivity. Immunohistochemical identification can provide a useful adjunct to the latter, and it has the capability to increase both sensitivity and specificity.

Monoclonal antibodies (MoAbs) are potentially of use in both serodiagnosis (in the definition, characterization and purification of antigens for use in antibody detection and in the direct development of antigen detection assays) and in immunohistochemistry. To a degree MoAbs have tended to replace existing polyclonal sera in diagnostic tests, a development which has arisen from the appreciation of the problems inherent in the use of polyclonal antisera, namely: a) batch variation between serum samples b) availability of limited stocks of sera, c) lack of standardisation between various antibody batches, with concurrent inability to compare the results of detection experiments, d) the relative lack of sensitivity of the antisera, e) difficulties in identifying the actual antigen being detected, f) the potential lack of specificity of such reagents, necessitating cross-absorption. In contrast the supply of any given MoAb is effectively unlimited, and

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each MoAb possesses defined chemical reproducibility and specificity. In addition, potentially such reagents will have greater sensitivity due to lower attendant background values, and their production does not require access to a continous supply of antigen.

That is not to say that the use of MoAbs is problem free, and there are potential pitfalls in the application of these reagents due to intrinsic cross reactivity resulting from the presence of related chemical structures or shared determinants. Other problems may occur as a result of low antibody affinity, and also arise due to low sensitivity resulting from a narrow range of reactivity. It will become evident during the course of this article that despite such potential limitations MoAbs have had a significant impact on the diagnosis of mycoses, and recent developments, particularly with regard to *Histoplasma capsulatum* and *Paracoccidioides brasiliensis* in particular suggest that the application of these reagents still has a great deal to offer.

***Aspergillus* sp.** The definitive diagnosis of aspergillosis takes into account data from clinical, radiological, serological and histopathological sources, and it is in regard to the last two areas that MoAbs have a role to play. It should be noted, however that with the exception of the invasive form of the disease, the diagnosis of aspergillosis is not generally difficult. In the case of invasive disease it is of particular concern that diagnosis is made without delay, since prognosis rapidly worsens in the absence of recognition and effective treatment.

A great deal of effort has gone into the detection of specific *Aspergillus fumigatus* antibodies in patients with allergic aspergillosis and aspergilloma [1-3] and MoAbs have played a role in the characterisation of the immunoreactive antigens which are central to such approaches. Although probably less useful diagnostically, the detection of antibody responses in individuals with invasive aspergillosis has also been attempted, and MoAbs have also played a useful role in antigen characterisation in this area [4,5].

However, in the context of invasive aspergillosis, and the patient groups who develop this form of the disease, the detection of antigen is conceptually a more effective means of diagnosis than antibody detection can ever be. Most interest has centered around the detection of galactomannan (GM) [6,7]. Until relatively recently the detector antibodies used were either polyclonal rabbit or human anti-*Aspergillus* antibodies [8-11]. However MoAbs have now been successfully used in the detection of GM, and they form the basis of commercially available kits. For example the Sanofi-Diagnostic Pasteur kit (France) relies on MoAbs directed against a tetra β(1-5) galactofuranoside [12], which appears to be the immunodominant epitope in GM. Various test formats have been used including latex agglutination [13,14], inhibition ELISA [11] and sandwich ELISA [15]; the latter offers the greatest sensitivity [7,16], with detectable GM levels of 0.5 to 1.0 ng/ml of sera [15]. Overall the sensitivity of the sandwich ELISA appears to be between 67-100%, with some patients having detectable GM in serum even before clinical indications of *Aspergillus* infection [15-18]. There is some limited evidence that the clinical outcome of disease correlates with the levels of detectable GM [17,19] and the ELISA can also be used with bronchoalveolar lavage samples [7,20]. It is noteworthy, however, that the generation of false positive results may still be a problem when detecting GM, with a rate of up to 15% reported recently [21]. Part of this problem may be due to a genuine cross-reaction with other fungi (particularly members of the genus *Penicillium* [21]).

The only other *Aspergillus* antigen which has been shown to be detectable in body fluids via the use of MoAbs is the 18kDa ribonuclease antigen [22,23]. A sandwich ELISA has been developed which combines either a mouse or rat MoAb with a rabbit polyclonal and which claims a sensitivity down to 1ng [7]. However, this test does not yet appear to have been commercialised.

Immunohistochemical identification of *Aspergillus* sp. *in situ* is potentially also a useful tool in the diagnosis of aspergillosis, and several reports deal with the application of MoAbs in this area [24-26]. These studies have typically used MoAbs directed against GM; it is worth noting, however, that in one such report it was concluded that immunoperoxidase staining using anti-GM antibody EB-A1 was no better than conventional histopathology in identifying the presence of infection. There is therefore scope for improvement in this area, possibly by the production of MoAbs which are directed at epitopes other than those present on GM.

***Candida* sp.** Invasive candidiasis is by far the most important and prevalent deep-seated fungal infection, and although microscopy and culture are important tools in diagnosis, there has been, and continues to be, considerable scope for the use of methods which rely on serodiagnostic detection. A wide range of techniques have been developed for the detection of anti-*Candida* antibodies, some of which have been commercialised [27,28]. These tests have some diagnostic value [28-31] and a number of immunodominant antigens have been investigated, including enolase [32-34]. The principle role of MoAbs in this area has been as tools in the definition and characterisation of such antigens [35,36]. However, all systems for the detection of anti-*Candida* antibodies are dependent on distinguishing between the normal titres found in healthy individuals who are exposed to *Candida* on a daily basis, and the hopefully elevated titres found in patients with symptomatic disease. There is also the additional problem that the presence of increased levels of antibodies does not necessarily distinguish between life threatening candidiasis and transitory infection which is of only moderate significance, unless the target antigen is one that is expressed solely in deep-seated candidiasis.

Given such circumstances a great deal of interest has focused on the detection of circulating *Candida* antigen. Mannan was the first of such antigens to be intensively investigated [37,38], and a commercial latex agglutination test (Pastorex Candida assay, Sanofi-Pasteur, France) which utilised a anti-mannan MoAb [39] was subsequently produced. This test is reactive to mannan from most of the commonly occurring species of *Candida*, with the notable exception of *Candida krusei.* However the sensitivity of the test is not perhaps all that it should be, and a recent study [40] revealed that antigen was detectable in only 25.6% of patients with candidemia. MoAbs have been made against the *Candida albicans* acid proteinase [41] although their use in the detection of this antigen has been handicapped by low sensitivity [42]. In contrast the detection of enolase has proved more effective [40] and the combination of a mouse MoAb with rabbit polyclonal was used as early as 1991 to detect candidiasis in a prospective study in patients with cancer [33]. Finally a MoAb against mannoprotein has also been used to detect antigen in a dot immunobinding format [43], although this approach does not appear to have been taken up commercially.

The Cand-Tec test, which detects a heat labile anti-

gen, has also been extensively employed, although this relies on a polyclonal antibody [44]. The effectiveness of this assay is apparently variable [40,45] although it may be useful in identifying those individuals likely to develop deep candidiasis [28]. The detection of β-glucan is also potentially diagnostically useful, although tests for this polysaccharide do not utilise MoAbs [46,47].

It should be evident from the preceding discussion that although a substantial number of antigen detection tests have been developed for candidiasis, all of them have certain limitations, particularly with regard to sensitivity and specificity. Their use in combination has been advocated to compensate for these shortcomings [40]. The impact of MoAbs on these assay systems is perhaps not as great as it might have been, and it would appear that there is still scope for deployment of these reagents in this field.

Polyclonal antibodies have proved of some use in the immunohistochemical diagnosis of candidiasis [48] and MoAbs have also been shown to have some potential in this area [49-51]. MoAb 1B12 [52] appears to be of particular value, with its reported ability to recognise *Candida* in paraffin-embedded tissue and its lack of reactivity to other fungi. In our experience, a sizeable percentage of MoAbs will not retain reactivity against fungi in paraffin embedded tissue due to antigenic denaturation during processing. Indeed this constitutes perhaps the biggest drawback to the application of MoAbs to this area and it is generally the exception, rather than the rule, to find MoAbs that are as reactive against paraffin embedded material as they are against frozen, lightly fixed material. Finally, it is of note that the application of MoAbs to the identification of *Candida* has also been extended to the direct and rapid identification of yeast colonies, with a commercial kit (the Bichro-latex albicans test, Fumouze, France) providing a useful tool [53,54]. This type of application for specific MoAbs has not generally been exploited in the identification of other medically important fungi, which is perhaps surprising given the potential time saving that might be made.

***Cryptococcus neoformans.*** Historically the identification of *C. neoformans* infections has relied on a combination of culture of CSF or the demonstration of encapsulated yeast cells in India ink preparations of CSF. However, the detection of circulating cryptococcal antigen in body fluids has now become probably the most effective and sensitive means of diagnosis [55-57]. Initially the antigen detection system relied on a latex agglutination system in which polyclonal antibodies directed against capsular components were used to coat latex particles [58,59]. This system generally proved itself to be both sensitive and specific, although there were some reports of problems associated with false positivity [57,60]. Subsequently the generation of MoAbs against capsular polysaccharide [61,62] offered scope for the development of tests that did not rely on polyclonal antibodies, and considerable effort has been expended in this area. The existing latex agglutination format has been modified to include MoAbs, with several commercial systems being produced in the early 1990s. These include the Pastorex Cryptococcus test (Sanofi-Pasteur), which when compared to agglutination tests which incorporated polyclonal antibodies, was found to be similarly sensitive and effective [63] and the CRYPTO-LEX (Trinity Laboratories, Inc., USA) [64], which was also found to be highly effective.

ELISA based systems are potentially more sensitive than those using latex agglutination and a number of variations have been developed. These include a two-site non-competitive ELISA that utilises the combination of a polyclonal antibody for antigen capture with a glucuronoxylomannan (GXM) binding MoAb [65] for antigen detection (the PREMIER Cryptococcal Antigen assay, Meridian Diagnostics, Inc., USA [66]). An experimental sandwich ELISA using combinations of IgG and IgM MoAbs [67] has also been used to define the importance of antibody isotype in the effectiveness of antigen capture.

MoAbs have also been produced against non capsule components [68] and one of these (the 115kDa glycoprotein) has been shown to elicit an antibody response [69]. However, antibody detection as a means of diagnosing *C. neoformans* infections comes in a poor second to antigen detection, and developments in this area have been restricted. It is of note that although some of these non-capsular reactive MoAbs [68] were able to label yeast cells by immunofluorescence antibody test (IFAT) on methanol fixed cryostat sections none of them has subsequently proved useful in identifying yeasts in paraffin wax embedded biopsy material. Indeed there remains a need for a *C. neoformans* specific MoAb for use in such situations, although the microscopic identification of *C. neoformans* in tissue is not as problematic as that of other fungal pathogens, largely because of its highly visible capsule. However, the application of polyclonal antibodies in this area [48,70] demonstrates that there is at least some potential in immunohistochemical diagnosis of *C. neoformans*.

***Blastomyces dermatitidis.*** Serodiagnosis of *B. dermatitidis* has historically relied on the detection of antibody responses against various antigens, by a variety of techniques [71,72] including a commercialised enzyme immunoassay [73]. Bradsher [74,75] has described the serodiagnosis of blastomycosis via antibody detection as problematic, largely because of limitations relating to low sensitivity and low specificity, although one of the more recently described radioimmunoassays claims a sensitivity of 85% and specificity approaching 100% [76]. MoAbs have not played a major role in the elucidation of the antigens used in such assays, nor have they been incorporated in the as yet limited attempts at antigen detection [77,78].

In terms of immunohistochemical diagnosis polyclonal antibodies have been used with some success [48], and those directed against the 120kDa yeast antigen [76] may be broadly applicable in this area. Thus far there are no reports in the literature dealing with the application of MoAbs to the immunohistochemical diagnosis of *B. dermatitidis*.

***Coccidioides immitis.*** A great deal of interest has focused on the detection of humoral responses against *C. immitis*, and a number of commercial kits have been made available to detect antibodies [28,79,80]. Generally antibody responses in *C. immitis* are a valuable aid both diagnostically and prognostically [81,82] and recombinant antigens have now been produced for use in a number of antibody detection systems which promise high sensitivity and specificity [83-85]. The major role of MoAbs has come in the characterization and definition of these antigens [86-88].

There have been some attempts to utilise antigen detection assays, which have relied on polyclonal antibodies; such assays have met with mixed success [89-91]. To date MoAbs have not been used in antigen detection, partly, perhaps, because the success of antibody detection methods has negated the development of such technology. Immunohistochemical diagnosis of *C. immitis* is depen-

dent on the production and use of polyclonal sera [48], and there are no reports on the use of MoAbs in this area.

***Sporothrix schenckii.*** Diagnosis of *S. schenckii* infection relies principally on clinical presentation combined with direct isolation/culture of the organism [92], although serology, via the detection of antibody responses has a role to play [93,94]. Polyclonal antibodies have also been used in immunohistochemical identification [95,96], with some success. There are few reports dealing with the production of MoAbs against *S. schenckii*, and those that are available relate to antigen characterisation [97]. There is probably no real demand for the development of MoAb based antigen detection ELISAs of the sort which have been recently developed for *H. capsulatum* and *P. brasiliensis*; however *S. schenckii* specific MoAbs would be useful in immunohistochemical identification of this fungus in clinical samples, and this remains a worthwhile area for study.

***Penicillium marneffei.*** Disseminated *P. marneffei* infections are relatively easy to diagnose because of the characteristic skin lesions which yield readily identifiable yeast forms [98,99]. The organism is also relatively easy to culture from clinical samples, and the characteristic red pigment produced by the mycelial form is also diagnostically useful [98,99]. However there remain obvious advantages to be gained from the early diagnosis of infection prior to the appearance of dermatological involvement. Research into the serodiagnosis of *P. marneffei* is still in its relative infancy, although several recent papers have identified a number of potentially useful antigens which may be recognised by patients sera [100-102]. Only one attempt has been made at antigen detection, involving the application of a rabbit polyclonal against fission arthroconidia filtrate [103].

Thus far the only report on the application of MoAbs to the diagnosis of *P. marneffei* infections relates to MoAb EB-A1, which recognises galactomannan in both *P. marneffei* and *Aspergillus* sp. [104]. This antibody has been used in the immunohistochemical identification of *P. marneffei* in formalin fixed specimens; however its lack of specificity is an obvious problem. A pre-absorbed polyclonal antibody has also demonstrated the potential for immunohistochemical detection [105]. There is thus clearly scope for the production and use of *P. marneffei* specific MoAbs as immunohistochemical tools, as reagents to define immunologically reactive antigens and as probes to use in antigen detection systems. Indeed efforts are currently underway in our laboratory to produce MoAbs against culture filtrate antigens of *P. marneffei* (specifically the 50,54 and 88kDa antigens [101]) and these reagents will, hopefully, have a significant impact on the diagnosis of *P. marneffei* infections.

***Histoplasma capsulatum.*** The diagnosis of histoplasmosis is based upon a combination of clinical observations and laboratory studies. Definitive diagnosis require growth of the fungus from samples of body fluids or tissues although this procedure may take up to four weeks to provide a positive result. Serological tests are important adjuncts to the diagnosis of histoplasmosis because of the difficulties inherent in detecting the organism by culture or by staining.

The most commonly used tests for the detection of anti-*H. capsulatum* antibodies are the complement fixation (CF) and immunoprecipitation methods which have been extensively reviewed elsewhere [106,107]. Precipitating antibodies detect two heat-sensitive glycoproteins (the M and H antigens) that are considered to be species specific [107]. Both the H and M antigen have been purified [108,109] although generally attempts to isolate these antigens have been concerned more with their serological activity than with their physiocochemical properties. MoAbs have played an important role in this area and have been used as an alternative method of purification because of the difficulty in purifying the M factor to homogeneity by methods based on size and charge. Two MoAbs of two different specifities were produced which permitted the characterisation of the M antigen with respect to its size, charge, and number of reactive components [110]. Other MoAbs have also been produced against the yeast antigen of *H. capsulatum* [111], which recognised epitopes of 62 and 72 kDa; however these reagents showed differing degrees of cross reactivity and no reports of subsequent attempts to use them in serodiagnosis were made. Hamilton *et al.* [112] used cyclophosphamide ablation to produce a MoAb with a high degree of species specificity which was able to differentiate between *H. capsulatum* var *capsulatum* and *H. capsulatum* var *duboisii* [113]. Unfortunately this reagent did not prove effective in the development of a serological test. Finally a MoAb was also raised against a 80kDa heat shock protein (hsp) of *H. capsulatum* [114]. Although this antigen has been shown to mediate protective immunity in mice it is not useful in serodiagnosis because in common with other heat shock proteins (hsps) it shows high homology with equivalent proteins from other dimorphic fungi.

Antigen detection has played an important role in serodiagnosis particularly since antibody titers may be very low or even absent in immunocompromised patients. A radioimmunoassay (RIA) developed in the mid 1980s [115] for the detection of a polysaccharide antigen using a rabbit polyclonal antibody has proved very useful especially in immunocompromised patients [116]. Forjtasek *et al.* [117] reported an attempt to produce MoAbs which could replace the polyclonal antibody used in the RIA. The two monoclonals produced were able to detect antigen in a small percentage of patients with disseminated histoplasmosis who had positive radioimmunoassays results although the MoAbs were considered unsuitable for replacing the rabbit polyclonal. More recently, an inhibition ELISA using a species specific MoAb has been applied to the detection of a 69-70 kDa circulating antigen. This assay appears to be both sensitive and specific [118]. More studies are needed to evaluate the usefulness of this new test in the follow-up of patients.

Immunohistochemistry is especially useful for the diagnosis of *H. capsulatum* infections because small cells of *H. capsulatum* var *capsulatum* may easily be confused with *Pneumocystis carinii, Candida* spp., *C. neoformans, P. marneffei* and *S. schenckii*. Polyclonal antibodies have been used although they have some limitations [48]. MoAbs raised against the histoplasmin antigens have been used but have been proved to be ineffective when applied in immunochemical assays. For example MoAbs against the purified M antigen have been found to intensively stain the yeast cells of both *H. capsulatum* var. *capsulatum* and *B. dermatitidis* [95] The anti-hsp80 MoAb [114] is also of no use in this context because of cross reactivity. Efforts are currently underway to use the new H1C MoAb [118] in immunohistochemistry.

***Paracoccidioides brasiliensis.*** The definitive diagnosis of paracoccidioidomycosis can only be accomplished by laboratory procedures such as direct examina-

tion, culture and serodiagnosis [119]. To date, no serological method for the diagnosis of paracoccidioidomycosis has been made commercially available, despite the fact that serological procedures are of great value [120]. Many different tests have been employed for antibody detection and immunodiffusion and complement fixation are the most widely employed. Historically the majority of these tests suffered from cross-reactivity. Polyclonal antibodies have been utilised in antigen characterization, and have also been used in antigen detection [120,121]. The first report detailing the production of species specific MoAbs used a modification of the standard MoAb technology [122] although these MoAbs were not subsequently used in serodiagnosis. MoAbs produced against the gp43 antigen, which is considered to be the most diagnostically useful antigen [119], have been applied in an capture enzyme immunoassay for the detection of specific human anti-gp43 immunoglobulin G in patients with paracoccidioidomycosis [123]. Using this assay the reactivities of patient sera were found to be significantly higher than those of control sera. More anti-gp43 MoAbs were produced and used to conduct a detailed study on the subcellular distribution of this antigen by immunoelectron microscopy [124]; these reagents were also used for *in vivo* studies to modulate laminin-mediated fungal adhesion to epithelial cells and pathogenesis [125], but have not, as yet, been used in serodiagnosis.

Several other *P. brasiliensis* antigens have now also been identified and characterized to a greater or lesser extent using MoAbs. These include the 58 kDa [126] and 22 kDa glycoproteins [127]. The former was recognized by 81% of immune human sera when tested by Western blot and the latter is of more interest as an immunohistochemical marker. More recently, an inhibition ELISA which utilises a MoAb (P1B) directed against an 87 kDa antigen [128] has been applied to the detection of circulating antigen in sera. The sensitivity of this assay is quite high (80.4%), although important cross reactions were also observed. The same MoAb has also been used in the inh-ELISA for the follow up of patients during treatment and results demonstrate that it constitutes an important tool for this purpose [129].

**Concluding remarks.** It should be apparent from the preceding review that the application of MoAbs has substantially added to our ability to diagnose systemic mycoses, particularly in regard to the detection of *Aspergillus*, *Cryptococcus*, *Histoplasma* and *Paracoccidioides* antigens in samples from patients. Antigen detection offers advantages over antibody detection, a factor which has largely been responsible for the growth in interest in this area. That is not to say that antigen detection offers a universal panacea to the serological diagnosis of disseminated mycoses, and substantial problems may be encountered relating to immune complex formation, rapid clearance of antigen and indeed the release of low or very low quantities of antigen. Some of these problems might be overcome by the use of mixtures of specific MoAbs directed against different determinants, an approach that as yet has received little attention.

In terms of the actual production of MoAbs great care should be taken in ensuring that they are made against antigens that are actually serodiagnostically useful. For example there are now a substantial number of reports on the production of MoAbs against fungal antigens, and yet comparatively few of these reagents, at least until relatively recently, have been used to produce useful assays. The impact of MoAbs in terms of immunohistochemical diagnosis has been much less profound, and much remains to be done in this area. Finally note should also be made with regard to the diagnosis of mycoses of the potential of the newer molecular biological based technologies, such as PCR based diagnosis, to augment or supercede serodiagnostic/immunohistochemical approaches. However, as yet the use of such methodologies is not generalized and as such there is still potential for an expansion of the application of MoAbs in the field of mycological diagnosis.

## References


1. DeRepentigny L, Reiss E. Current trends in immunodiagnosis of candidiasis and aspergillosis. Rev Infect Dis 1984;6:301-312.
2. Hearn VM. Serodiagnosis of aspergillosis. In:Vanden Bossche H, Mackenzie DWR, Cauwenbergh G (Eds) *Aspergillus*, Aspergillosis. New York, Plenum, 1988: 43-71.
3. Hamilton AJ, Holdom MD, Hay RJ. Specific recognition of purified Cu, Zn superoxide dismutase from *Aspergillus fumigatus* by immune human sera. J Clin Microbiol 1995;33:495-496.
4. Burnie JP, Matthews RC. Heat shock protein 88 and *Aspergillus* infection. J Clin Microbiol 1991;29:2099-2106.
5. Fratamico PM, Buckley HR. Identification and characterization of an immunodominant 58-kilodalton antigen of *Aspergillus fumigatus* recognized by sera of patients with invasive aspergillosis. Infect Immun 1991;59:309-315.
6. Reiss E, Lehmann PF. Galactomannan antigenemia in invasive aspergillosis. Infect Immun 1979;25:357-365.
7. Latge JP. Tools and trends in the detection of *Aspergillus fumigatus*. Curr Top Med Mycol 1995;6: 245-281.
8. Schaffer PJ, Medoff G, Kobayashi GS. Demonstration of antigenemia in patients with invasive aspergillosis by solid phase (protein A-rich *Staphylococcus aureus*) radioimmunoassay. Am J Med 1979;67:627-630.
9. Weiner MH, Coats-Stephen M. Immunodiagnosis of systemic aspergillosis I: antigenemia detected by radioimmunoassay in experimental infection. J Lab Clin Med 1979;93:111-119.
10. Johnson TM, Kurup VP, Resnick A, Ash RC, Fink JN, Kalbfleisch J. Detection of circulating *Aspergillus fumigatus* antigen in bone marrow transplant patients. J Lab Clin Med 1989;114:700-707.
11. Rogers TR, Haynes KA, Barnes RA. Value of antigen detection in predicting invasive pulmonary aspergillosis. Lancet 1990;336:1210-1213.
12. Stynen D, Sarfati J, Goris A, *et al.* Rat monoclonal antibodies against *Aspergillus* galactomannan. Infect Immun 1992; 60:2237-2245.
13. Dupont B, Improvisi L, Provost F. Detection de galactomannane dans les aspergilloses invasives humaines et animales avec un test au latex. Bull Soc Fr Mycol Med

1990;19:35-42.
14. Haynes KA, Rogers TR. Retrospective evaluation of a latex agglutination test for diagnosis of invasive aspergillosis in immunocompromised patients. Eur J Clin Microbiol Infect Dis 1994; 13:670-674.
15. Stynen D, Goris A, Sarfati J, Latge JP. A new sensitive sandwich enzyme-linked immunosorbent assay to detect galactofuran in patients with invasive aspergillosis. J Clin Microbiol 1995;33:497-500.
16. Sulahian A, Tabouret M, Ribaud P, *et al.* Comparison of an enzyme immunoassay and latex agglutination test for detection of galactomannan in the diagnosis of invasive aspergillosis. Eur J Clin Microbiol Infect Dis 1996;15:139-145.
17. Verweij PE, Stynen D, Rijs AJJM, *et al.* Sandwich enzyme linked-immunosorbent assay compared with Pastorex latex agglutination test for diagnosing invasive aspergillosis in immunocompromised patients. J Clin Microbiol 1995;33:1912-1914.
18. Rohrlich P, Sarfati J, Mariani P *et al.* Prospective sandwich enzyme-linked immunosorbent assay for serum galactomannan: early predictive value and clinical use in invasive aspergillosis. Pediatr Infect Dis J 1996;15:232-237.
19. Verweij PE, Dompeling EC, Donnelly JP, Schattenberg AVMB, Meis JGFM. Serial monitoring of *Aspergillus* antigen in the early diagnosis of invasive aspergillosis. Preliminary investigations with two examples. Infection 1997; 25:86-89.
20. Verweij PE, Latge JP, Rijs AJMM, *et al.* Comparison of antigen detection and PCR assay using bronchoalveolar lavage fluid for diagnosing invasive pulmonary aspergillosis in patients receiving treatment for hematological malignancies. J Clin Microbiol 1995;33:3150-3153
21. Swanink CMA, Meis JFGM, Rijs AJMM, Donnelly JP, Verweij PE. Specificity of a sandwich enzyme-linked immunosorbent assay for detecting *Aspergillus* galactomannan. J Clin Microbiol 1997;35:257-260.
22. Lamy B, Davies J. Isolation and nucleotide sequence of the *Aspergillus restrictus* gene coding for the ribonucleolytic toxin restrictocin and its expression in *A. nidulans*: the leader sequence protects producing strains from suicide. Nucl Acids Res 1991;19:1001-1006.
23. Lamy B, Moutaouakil M, Latge JP, Davies J. Secretion of a potential virulence factor, a fungal ribonucleotoxin, during aspergillosis infections. Mol Microbiol 1991;5:1811-1815.
24. Arrese Estrada J, Stynen D, Goris A, Pierard GE. Immunohistochemical identification of *Aspergillus* by monoclonal antibody EB-A1. Ann Pathol 1990;10:198-200.
25. Jensen HE, Aalbaek B, Lind P, Frandsen PL, Krogh HV, Stynen D. Enzyme immunohistochemistry with mono- and polyclonal antibodies in the pathological diagnosis of systemic bovine mycoses. APMIS 1993;101:505-516.
26. Verweij PE, Smedts F, Poot T, *et al.* Immunoperoxidase staining for identification of *Aspergillus* species in routinely processed tissue sections. J Clin Pathol 1996;49:789-801.
27. Gutierrez J, Maroto C, Piedrola G, Martin E, Perez JA. Circulating *Candida* antigens and antibodies: useful markers of candidemia. J Clin Microbiol 1993;31:2550-2552.
28. Kappe R, Seeliger HPR. Serodiagnosis of deep-seated fungal infections. Curr Top Med Mycol 1993;5:247-280.
29. Kobayashi K, Mukae N, Matsunaga Y, Hotchi M. Diagnostic value of serum antibody to *Candida* in an extensively burned patient. Burns 1990; 16:414-417.
30. Zoller L, Kramer I, Kappe R, Sonntag HG. Enzyme immunoassays for invasive *Candida* infections: reactivity of somatic antigens of *Candida albicans*. J Clin Microbiol 1991;29:1860-1867.
31. Garcia-Ruiz JC, Arilla MC, Regulez P, Quindos G, Alvarez A, Ponton J. Detection of antibodies to *Candida albicans* germ tubes for diagnosis and therapeutic monitoring of invasive candidiasis in patients with haematological malignancies. J Clin Microbiol 1997;35:3284-3287.
32. Franklyn KM, Warmington JR, Ott AK, Ashman RB. An immunodominant antigen of *Candida albicans* shows homology to the enzyme enolase. Immunol Cell Biol 1990;68:173-178.
33. Walsh TJ, Hathorn JW, Sobel JD, *et al.* Detection of circulating *Candida* enolase by immunoassay in patients with cancer and invasive candidiasis. N Engl J Med 1991;324:1026-1031.
34. van Deventer AJ, van Vliet HJ, Hop WC, Goessens WH. Diagnostic value of anti-*Candida* enolase antibodies. J Clin Microbiol 1994;32:17-23.
35. Sundstrom PM, Tam MR, Nichols EJ, Kenny GE. Antigenic differences in the surface mannoproteins of *Candida albicans* as revealed by monoclonal antibodies. Infect Immun 1988;56:601-606.
36. Stockbine NA, Largen MT, Buckley HR. Production and characterisation of three monoclonal antibodies to *Candida albicans* proteins. Infect Immun 1984;43:1012-1018.
37. De Repentigny L, Kaufman L, Cole GT, Kruse D, Latge JP, Mathews RC. Immunodiagnosis of invasive fungal infections. J Med Vet Mycol 1994;32 (Suppl 1): 239-252.
38. Buckley HR, Richardson MD, Evans EGV, Wheat LJ. Immunodiagnosis of invasive fungal infections. J Med Vet Mycol 1992;30 (Suppl. ):249-260.
39. Herent P, Stynen D, Hernando F, Fruit J, Poulain D. Retrospective evaluation of two latex agglutination tests for detection of circulating antigens during invasive candidosis. J Clin Microbiol 1992;30:2158-2164.
40. Mitsutake K, Miyazaki T, Tashiro T, *et al.* Enolase antigen, mannan antigen, Cand-Tec antigen, and beta-glucan in patients with candidemia. J Clin Microbiol 1996;34:1918-1921.
41. Borg M, Watters D, Reich B, Ruchel R. Production and characterization of monoclonal antibodies against secretory proteinase of *Candida albicans* CBS 2730. Zentralbl Bakteriol Mikrobiol Hyg A 1988;268:62-71.
42. Ruchel R. Proteinase. In: Bennett JE, Hay RJ, Peterson PK (Eds) New strategies in fungal diseases. Edinburgh, Churchill-Livingstone, 1992: 17-31.
43. DeBernardis F, Girmenia C, Boccanera M, Adriani D, Martino P, Cassone A. Use of a monoclonal antibody in a dot immunobinding assay for detection of a circulating mannoprotein of *Candida* spp. in neutropenic patients with invasive candidiasis. J Clin Microbiol 1993;31:3142-3146.
44. Fung JC, Donta ST, Tilton RC. *Candida* detection system (CAND-TEC) to differentiate between *Candida albicans* colonization and disease. J Clin Microbiol 1986;24:542-547.
45. Phillips P, Dowd A, Jewesson P, *et al.* Nonvalue of antigen detection immunoassay for diagnosis for candidemia. J Clin Microbiol 1990; 28:2320-2326.
46. Obayashi T, Yoshida M, Mori T, *et al.* Plasma (1-3)-beta-D-glucan measurement in diagnosis of invasive deep mycosis and fungal febrile episodes. Lancet 1994;345:17-20.
47. Miyazaki T, Kohno S, Mitsutake K, *et al.* Plasma (1-3)-beta-D-glucan and fungal antigenemia in patients with candidemia, aspergillosis, and cryptococcosis. J Clin Microbiol 1995;33:3115-3118.
48. Jensen HE, Schønheyder HC, Hotchi M, Kaufman L. Diagnosis of systemic mycoses by specific immunohistochemical tests. APMIS 1996;104:241-258.
49. Hopwood V, Poulain D, Fortier B, Evans G, Vernes A. A monoclonal antibody to a cell wall component of *Candida albicans*. Infect Immun 1986; 54:222-227.
50. Cailliez JC, Boudrissa A, Mackenzie DWR, Poulain D. Evaluation of a gold-silver staining method for detection and identification of *Candida* species by light microscopy. Eur J Clin Microbiol Infect Dis 1990; 9:886-891.
51. Breier F, Oesterreicher C, Brugger S, Linkesch W, Anegg B, Jurecka W. Immunohistochemistry with monoclonal antibody against *Candida albicans* mannan antigen demonstrates cutaneous *Candida* granulomas as evidence of *Candida* sepsis in an immunosuppressed host. Dermatology 1997;194:293-296.
52. Monteagudo C, Marcilla A, Mormeneo, Llombart-Bosch A, Sentandreu R. Specific immunohistochemical identification of *Candida albicans* in paraffin-embedded tissue with a new monoclonal antibody (1B12). Am J Clin Pathol 1995;103:130-135.
53. Robert R, Sentandreu R, Bernard C, Senet J-M. Évaluation du réactif Biochrolatex *albicans* pour l'identification de colonies de *Candida albicans*. J Mycol Med 1994; 4:226-229.
54. Freydiere AM, Buchaille L, Guinet R, Gille Y. Evaluation of latex reagents for rapid identification of *Candida albicans* and C. krusei colonies. J Clin Microbiol 1997;35:877-880.
55. Eng RHK, Bishburg E, Smith SM, Kapila R. Cryptococcal infections in patients with acquired immune deficiency syndrome. Am J Med 1986; 81:19-23.
56. Chuck SL, Sande MA. Infections with *Cryptococcus neoformans* in the acquired immunodeficiency syndrome. N Engl J Med 1989; 321:794-799.
57. Heelan JS, Corpus L, Kessimian N. False-positive reactions in the latex agglutination test for *Cryptococcus neoformans* antigen. J Clin Microbiol 1991; 29;1260-1261
58. Wu TC, Koo SY. Comparison of three commercial cryptococcal latex kits for detection of cryptococcal antigen. J Clin Microbiol 1983; 18:1127-1130.
59. Zuger A, Louie E, Holzman RS, Simberkoff MS, Rahal JJ. Cryptococcal disease in patients with the acquired immunodeficiency syndrome. Diagnostic features and outcome of treatment. Ann Intern Med 1986; 104:234-240.
60. Hay RJ, Mackenzie DWR. False-positive latex tests for cryptococcal antigen in cerebrospinal fluid. J Clin Microbiol 1982; 35:244-245.
61. Dromer F, Salamero J, Contrepois A, Carbon C, Yeni P. Production,characterization and antibody specificity of a mouse monoclonal antibody reactive with *Cryptococcus neoformans* capsular polysaccharide. Infect Immun 1987;55:742-748.
62. Todaro-Luck F, Reiss E, Cherniak R, Kaufman L. Characterization of *Cryptococcus neoformans* capsular glucuronoxylomannan polysaccharide with monoclonal antibodies. Infect Immun 1989;57:3882-3887.
63. Temstet A, Roux P, Poirot JL, Ronin O, Dromer F. Evaluation of a monoclonal antibody-based latex agglutination test for diagnosis of cryptococcosis: comparison with two tests using polyclonal antibodies. J Clin Microbiol 1992;30:2544-2550.
64. Kiska DL, Orkiszewski DR, Howell D, Gilligan PH. Evaluation of new monoclonal antibody-based latex agglutination test for detection of cryptococcal polysaccharide antigen in serum and cerebrospinal fluid. J Clin Microbiol 1994; 32;2309-2311.
65. Eckert TF, Kozel TR. Production and characterization of monoclonal antibodies specific for *Cryptococcus neoformans* capsular polysaccharide. Infect Immun 1987;55:1895-1899.
66. Gade W, Hinnefeld SW, Babcock LS, *et al.* Comparison of the Premier cryptococcal antigen enzyme immunoassay and the latex agglutination assay for detection of cryptococcal antigens. J Clin Microbiol 1991; 29:1616-1619.
67. Mukherjee S, Casadevall A. Sensitivity of sandwich enzyme-linked immunosorbent assay for *Cryptococcus neoformans* polysaccharide antigen is dependent on the isotypes of the capture and detection antibodies. J Clin Microbiol 1995; 33:765-768.
68. Hamilton AJ, Jeavons L, Hobby P, Hay RJ. A 34- to 38-kilodalton *Cryptococcus neoformans* glycoprotein produced as an exoantigen bearing a glycosylated species-specific epitope. Infect Immun 1992; 60:143-149.
69. Hamilton AJ, Goodley J. Purification of the 115-kilodalton exoantigen of *Cryptococcus neoformans* and its recognition by immune sera. J Clin Microbiol 1993;31:335-339.
70. Kaplan W, Bragg SL, Crane S, Ahearn

DG. Serotyping *Cryptococcus neoformans* by immunofluorescence. J Clin Microbiol 1981; 14:313-317.
71. Klein BS, Kuritsky JN, Chapell WA, *et al.* Comparison of the enzyme immunoassay, immunodiffusion, and complement fixation tests in detecting antibody in human serum to the A antigen of *Blastomyces dermatitidis*. Amer Rev Resp Dis 1986;133:144-148.
72. Klein BS, Vergeront JM, Kaufman L, *et al.* Serological tests for blastomycosis: assessments during a large point-source outbreak in Wisconsin. J Infect Dis 1987; 155:262-268.
73. Sekhon AS, Kaufman L, Kobayashi GS, Moledina NH, Jalbert M. The value of the Premier enzyme immunoassay for diagnosing *Blastomyces dermatitidis* infections. J Med Vet Mycol 1995;33:123-125.
74. Bradsher RW. A clinician's view of blastomycosis. Curr Top Med Mycol 1993;5:181-200.
75. Bradsher RW. Histoplasmosis and blastomycosis. Clin Infect Dis 1996;22,(Suppl. 2): S102-S111.
76. Klein BS, Jones JM. Isolation, purification and radiolabelling of a novel 120-kD surface protein on *Blastomyces dermatitidis* yeasts to detect antibody in infected patients. J Clin Invest 1990;85:152-161.
77. Lo CY, Notenboom RH. A new enzyme immunoassay specific for blastomycosis. Am Rev Respir Dis 1990; 141:84-88.
78. Areno JP, Campbell GD, George RB. Diagnosis of blastomycosis. Sem Resp Infect 1997;12:252-262.
79. Galgiani JN, Grace GM, Lundergan LL. New serologic tests for early detection of coccidioidomycosis. J Infect Dis 1991;163:671-674.
80. Martins TB, Jaskowski TD, Mouritsen CL, Hill HR. Comparison of commercially available enzyme immunoassay with traditional serological tests for detection of antibodies to *Coccidioides immitis*. J Clin Microbiol 1995;33: 940-943.
81. Pappagianis D, Zimmer BL. Serology of coccidioidomycosis. Clin Microbiol Rev 1990;3:247-268.
82. Yang MC, Magee DM, Cox RA. Mapping of a *Coccidioides immitis*-specific epitope that reacts with complement-fixing antibody. Infect Immun 1997;65:4068-4074.
83. Johnson SM, Zimmermann CR, Pappagianis D. Use of recombinant *Coccidioides immitis* complement fixation antigen-chitinase in conventional serological assays. J Clin Microbiol 1996;34:3160-3164.
84. Zimmermann CR, Johnson SM, Martens GW, White AG, Pappagianis D. Cloning and expression of the complement fixation antigen-chitinase of *Coccidioides immitis*. Infect Immun 1996;64:4967-4975.
85. Zhu Y, Tryon V, Magee DM, Cox RA. Identification of a *Coccidioides immitis* antigen 2 domain that expresses B-cell-reactive epitopes. Infect Immun 1997;65:3376-3380.
86. Dolan MJ, Cox RA, Williams V, Woolley S. Development and characterization of a monoclonal antibody against the tube precipitin antigen of *Coccidioides immitis*. Infect Immun 1989;57:1035-1039.
87. Cox RA, Dolan MJ, Magee DM, Galgiani JN. Production of a murine monoclonal antibody that recognises an epitope specific to *Coccidioides immitis* antigen 2. Infect Immun 1993; 61:1895-1899.
88. Zhu Y, Yang C, Magee DM, Cox RA. Molecular cloning and characterization of *Coccidioides immitis* antigen 2 cDNA. Infect Immun 1995;64:2695-2699.
89. Weiner MH. Antigenemia detected in human coccidioidomycosis. J Clin Microbiol 1983;18:136-142.
90. Galgiani JN, Dugger KO, Ito JI, Weiden MA. Antigenemia in primary coccidioidomycosis. Am J Trop Med Hyg 1984;33:645-649.
91. Wack EE, Dugger KO, Galgiani JN. Enzyme-linked immunosorbent assay for antigens of *Coccidioides immitis*: human sera interference corrected by acidification-heat extraction. J Lab Clin Med 1988;111:560-565.
92. Davis BA. Sporotrichosis. Dermatol Clin 1996; 14:69-76.
93. Aram H. Sporotrichosis, a historical approach. Int J Dermatol 1986;25:203-205.
94. Winn RE. A contemporary view of sporotrichosis. Curr Top Med Mycol 1995;6:73-94.
95. Kaufman L. Immunohistologic diagnosis of systemic mycoses: an update. Eur J Epidemiol 1992; 8:377-382.
96. Marques ME, Coelho KI, Sotto MN, Bacchi CE. Comparison between histochemical and immunohistochemical methods for diagnosis of sporotrichosis. J Clin Pathol 1992; 45:1089-1093.
97. Hamilton AJ, Harada T, Bartholomew MA, *et al.* Preparation of murine monoclonal antibodies against the yeast phase of the dimorphic fungus *Sporothrix schenckii*. Trans Roy Soc Trop Med Hyg 1990; 84:734-737.
98. Duong TA. Infection due to *Penicillium marneffei*, an emerging pathogen: review of 155 reported cases. Clin Infect Dis 1996;23:125-130.
99. Drouhet E. Penicilliosis due to *Penicillium marneffei*: a new emerging systemic mycosis in AIDS patients travelling or living in Southeast Asia. Review of 44 cases reported in HIV infected patients during the last 5 years compared to 44 cases of non AIDS patients reported over 20 years. J Mycol Med 1993;4:195-224.
100. Chongtrakool P, Chaiyaroj SC, Vithayasai V, *et al.* Immunoreactivity of a 38-kilodalton *Penicillium marneffei* antigen with human immunodeficiency virus-positive sera. J Clin Microbiol 1997;35:2220-2223.
101. Jeavons L, Hamilton AJ, Vanittanakom N, *et al.* Identification and purification of specific *Penicillium marneffei* antigens and their recognition by human immune sera. J Clin Microbiol 1998;36: 949-954.
102. Cao L, Chan CM, Lee C, Wong SS, Yuen KY. MP1 encodes an abundant and highly antigenic cell wall mannoprotein in the pathogenic fungus *Penicillium marneffei*. Infect Immun 1998;66:966-973.
103. Kaufman L, Standard PG, Jalbert M, *et al.* Diagnostic antigenemia tests for penicilliosis marneffei. J Clin Microbiol 1996;34:2503-2505.
104. Arrese Estrada J, Stynen D, Van Cutsem J, Pierard-Franchimont C, Pierard GE. Immunohistochemical identification of *Pennicilium marneffe*i by monoclonal antibody. Intern J Dermatol 1992; 31:410-412.
105. Kaufman L, Standard PG, Anderson SA, *et al.* Development of specific fluorescent-antibody test for tissue form of *Pennicilium marneffe*i. J Clin Microbiol 1995;33:2136-2138.
106. Bullock WE. *Histoplasma capsulatum.* In: Mandell GL, Bennet J, Dolin M (Eds. ) Principles and practice of infectious diseases. New York, Churchill Livingstone, 1995: 2340-2353
107. Kwon-Chung KJ and Bennet JE. Histoplasmosis. In: Kwon-Chung KJ and Bennet JE (Eds.). Medical Mycology. Lea & Febiger. Philadelphia London. 1992: 464-513.
108. Pine L. *Histoplasma* antigens: their production, purification and uses. Contrib Microbiol Immunol 1977;3:138-168.
109. Zancopé-Oliveria RM, Bragg SL, Hurst SF, Peralta JM, Reiss E. Evaluation of cation exchange chromatography for the isolation of M glycoprotein from histoplasmin. J Med Vet Mycol 1993;31:29-41.
110. Reiss E, Knowles JB, Bragg SL, Kaufman L. Monoclonal antibodies against the M-protein and carbohydrate antigens of histoplasmin characterized by the enzyme-linked immunoelectrotransfer blot method. Infect Immun 1986; 53: 540-546.
111. Kamel SM, Wheat LJ, Garten ML, Bartlett MS, Tansey MR, Tewari RP. Production and characterization of murine monoclonal antibodies to *Histoplasma capsulatum* yeast cell antigens. Infect Immun 1989; 57: 896-901.
112. Hamilton AJ, Bartholomew MA, Fenelon LE, Figueroa J, Hay RJ. A murine monoclonal antibody exhibiting high species specificity for *Histoplasma capsulatum* var. *capsulatum*. J Gen Microbiol 1990; 136:331-335.
113. Hamilton AJ, Bartholomew MA, Fenelon L, Figueroa J, Hay RJ. Preparation of monoclonal antibodies that differentiate between *Histoplasma capsulatum* variant *capsulatum* and *H. capsulatum* variant *duboisii*. Trans R Soc Trop Med Hyg 1990; 84: 425-428.
114. Jeavons L, Hunt L, Hamilton A. Immunochemical studies of heat-shock protein 80 of *Histoplasma capsulatum*. J Med Vet Mycol 1994; 32: 47-57.
115. Wheat LJ, Kohler R, Tewari R. Diagnosis of disseminated histoplasmosis by detection of *Histoplasma capsulatum* antigen in serum and urine specimens. N Engl J Med 1986; 314: 83-88
116. Wheat LJ, Connolly-Stringfield P, Blair R, Connolly K, Garringer T, Katz BP. Histoplasmosis relapse in patients with AIDS: detection using *Histoplasma capsulatum* variety *capsulatum* antigen levels. Ann Intern Med 1991; 115: 936-941.
117. Fojtasek MF, Kamel S, Karimi S, Connolly-Stringfield P, Wheat LJ. Monoclonal antibodies for detection of *Histoplasma capsulatum* variety *capsulatum* antigen in patients with histoplasmosis. Serodiag Immunother Infect Dis 1993; 5: 181-185
118. Gómez BL, Figueroa JI, Hamilton AJ, *et al.* Development of a novel antigen detection test for histoplasmosis. J Clin Microbiol 1997; 35: 2618-2622.
119. Brummer E, Castañeda E, Restrepo A. Paracoccidioidomycosis: an update. Clin Microbiol Rev 1993; 6:89-117.
120. Mendes Giannini MJS, Del Negro GB, Siqueira AM. Serodiagnosis. In: Franco M, Da Silva Lacaz C, Restrepo A (Eds.) Paracoccidioidomycosis. Boca Raton, CRC Press, 1994: 345-363.
121. Aparecida M, Shikanai-Yasuda MA, Poncio Mendes R, Barraviera B, Mendes Giannini MJS. Detection of circulating *Paracoccidioides brasiliensis* antigen in urine of paracoccidioidomycosis patients before and during treatment. J Clin Microbiol 1998; 36: 1723-1728.
122. Figueroa JI, Hamilton AJ, Bartholomew MA, Harada T, Fenelon L, Hay RJ. Preparation of species-specific murine monoclonal antibodies against yeast phase of *Paracoccidioides brasiliensis*. J Clin Microbiol 1990; 28: 1766-1769.
123. Camargo ZP, Gesztesi JL, Saraiva EC, Taborda CP, Vicentini AP, Lopes, JD. Monoclonal antibody capture enzyme immunoassay for detection of *Paracoccidioides brasiliensis* antibodies in paracoccidioidomycosis. J Clin Microbiol 1994; 32: 2377-2381.
124. Straus AH, Freymuller E, Travassos LR, Takahashi HK. Immunochemical and subcellular localisation of the 43 kDa glycoprotein antigen of *Paracoccidioides brasiliensis* with monoclonal antibodies. J Med Vet Mycol 1996; 34: 181-186.
125. Gesztesi JL, Puccia R, Travassos LR, *et al.* Monoclonal antibodies against the 43,000 Da glycoprotein from *Paracoccidioides brasiliensis* modulate laminin-mediated fungal adhesion to epithelial cells and pathogenesis. Hybridoma 1996, 15:415-422.
126. Figueroa JI, Hamiltom AJ, Allen MH, Hay R. Isolation and partial characterization of a *Paracoccidioides brasiliensis* 58 kDa extracellular glycoprotein which is recognized by human immune sera. Trans R Soc Trop Med Hyg 1995; 89: 566-572.
127. Figueroa JI, Hamilton A, Allen M, Hay R. Immunohistochemical detection of a novel 22-to 25-kDa glycoprotein of *Paracoccidioides brasiliensis* in biopsy material and partial characterization by using species-specific monoclonal antibodies. J Clin Microbiol 1994; 32: 1566-1574.
128. Gómez BL, Figueroa JI, Hamilton AJ, *et al.* Use of monoclonal antibodies in diagnosis of paracoccidioidomycosis: new strategies for detection of circulating antigens. J Clin Microbiol 1997; 35: 3278-3283.
129. Gómez BL, Figueroa JI, Hamilton AJ, *et al.* Antigenemia in paracoccidioidomycosis: detection of the 87 kDa determinant in patients during and after antifungal treatment. J Clin Microbiol 1998; submitted.