

SERION ELISA *classic*
Candida albicans IgG/IgM/IgA

1. ABSTRACT
2. INTRODUCTION
3. PATHOGEN
4. PATHOGENESIS
5. EPIDEMIOLOGY
6. CLINICAL FINDINGS
7. DIAGNOSIS
8. DESCRIPTION OF THE TEST
9. STUDIES
10. SUMMARY
11. DIAGRAMS
12. BIBLIOGRAPHY

1. ABSTRACT

- Increased diagnostic evidence by mannan-reduced antigen containing cytoplasmatic structures and cell-wall components
- differentiation and quantification of IgG-, IgA-, and IgM-antibodies
- good correlation of IgM-antibody kinetics with clinical findings
- detection of infections with non-candida albicans species (u.a. *C. glabrata*, *C. tropicalis*)

2. INTRODUCTION

During the past years an increasing number of mycosis with fatal outcome has been observed. The main reasons are (i) an increasing number of intensive patients, (ii) a broad spectrum of prophylactic given antibiotics, (iii) an optimized radio- and chemotherapy resulting in neutropenic phases of cancer patients, (iiii) and optimized surgical techniques resulting in a higher number of intensive patients with higher life expectance.

Infections with *Candida albicans* show a number of different clinical manifestations. The course of infection as well as the prognosis of candidosis is mainly influenced by the basic illness. In spite of a broad spectrum of different intensive medical treatments, systemic candida mycosis is often lethal, especially affecting haematooncological patients. Therefore, a rapid and reliable diagnosis at an early stage of the infection is of great importance.

3. PATHOGEN

Candida albicans is a ubiquitous occurring yeast, which belongs - like all *Candida ssp.* - to the family of yeast like fungi and is limited to humans as the only natural reservoir (table 1).

Table 1:

medically relevant fungi

1. yeast like fungi	<i>Candida ssp.</i> <i>Torulopsis</i> <i>Cryptococcus</i>
2. molds	<i>Aspergillus ssp.</i> <i>Mucor ssp.</i> <i>Rhizopus ssp.</i>
3. dermatophyte	
4. dimorphic fungi	

Besides the yeast form, which mainly occurs in superficial infections, the so-called pseudomycelium is another morphological form of yeast like fungi. The development of pseudomycelia and germ tubes is mostly limited to invasive mycosis. *Candida ssp.* produces and secretes several enzymes allowing this microorganism to penetrate blood vessels and mucosa.

4. PATHOGENESIS

Candida ssp. is principally transferred from human to human by smear infections. The main entrances of the pathogen are the mouth cavity and pharynx. In case of disorders of the fungistatic characteristics of the skin which may be caused by a light sour ph and free fatty acids produced by the normal fungi skin flora, superficial infections with *Candida ssp.* are likely to occur. Invasive mycosis may follow superficial infections of the mucosa mainly starting from the gastrointestinal tract as shown in the scheme below:

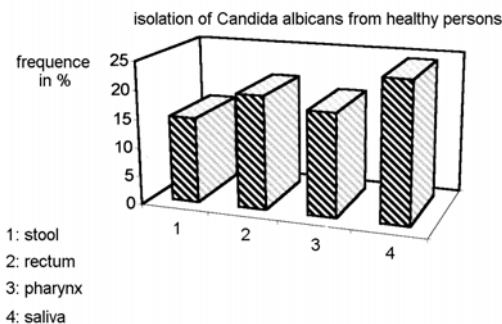
Adherence → Invasion → induction of immune defense

Specialized proteins and structures of the cell wall mediate attachment of yeast like fungi to human mucosa. For efficient infection of the host, *Candida ssp.* possesses a number of so called „evasion mechanisms“: 1. high frequency switching of the genome; 2. efficient attachment to host cells; 3. resistance mechanisms against antibiotics; 4.) escape mechanisms from the immune system.

5. EPIDEMIOLOGY

Candida ssp. is not supposed to belong to the permanent micro flora in healthy people. From time to time, repeated colonization of the healthy human host without any clinical findings are observed quite frequently as shown in table 1:

Diagram 1:



The organism is preferentially isolated from the intestinal tract and the mouth cavity by culture. Most infections occur by smear and contact infections transmission.

According to the localization of the pathogen, Candidosis is divided into two classes. The main features of these classes are listed in table 2:

Table 2:

	Infections with <i>Candida</i>	
	superficial	deep seated
Localization	Mucosa, skin	organs
status of the host's immune system	+	± / -
risk factors	pregnancy diabetes AIDS disorders of the skin	chemotherapy immunosuppressions intravenous catheters radiotherapy burns of higher degrees surgical intervention
clinical course	mild no danger to life	life-threatening
Therapy	++	+/-

Most commonly, the mouth cavity, the vaginal tract or the foot and fingernails are affected in superficial mycoses. *Candida* penetrates into the blood system and leaves the body via renal

secretion resulting in the possibility of a temporary isolation of this microorganism from urine samples. Even in cases of repeated chronic infections, clinical findings are rare and systemic therapy provides an efficient tool for eradication in the immunocompetent host.

In contrast, infections of the intestine are very likely to end up in a deep-seated candidosis in immunocompromized patients. In these cases, the degree of the granulocytopenia is a reliable indicator for an invasive mycosis.

In addition to immunocompromized, newborns and intensive patients with permanent catheters are also at high risk for a deep-seated candidosis.

6. CLINICAL FINDINGS

Infection with *Candida ssp.* shows a broad spectrum of clinical manifestations:

Table 3:

immunocompetent host	immunocompromized host
<ul style="list-style-type: none"> ▪ oral mycosis ▪ vaginal mycosis ▪ infections of the skin and the mucosa ▪ Onychomycosis 	<ul style="list-style-type: none"> ▪ urogenital mycosis (bladder, kidney) ▪ pulmonal mycosis ▪ infections of the CNS (encephalitis) ▪ endocarditis/ myocarditis ▪ peritonitis ▪ gastrointestinal mycosis ▪ hepatitis ▪ arthritic manifestations

Fever of „unknown origin“ is often the only criterion for the beginning of diagnosis and therapy in immunocompromized patients. When standardized antibiotics fail, a deep-seated candidosis seems likely.

While infections of the mouth cavity are facilitated by smoking, diabetes, AIDS or local disorders of the mucosa leading to a stomatitis, 50 % of infections of the oesophagus do not cause any symptoms. In 20 to 50 % of healthy adults, *Candida albicans* can be isolated from oropharynx specimens.

Clinical manifestations of a chronic infection of the stomach with *Candida* are not yet clear. An interesting feature thereby is the resistance of the microorganism against the acidic conditions in the stomach.

Infections of the Vagina are also facilitated by predispositions as pregnancy, diabetes, or systemic antibiotic therapy.

Systemic and hematogenous candidosis are mainly observed in cancer and transplantation patients. The risk for a candidosis depends on the localization of the transplanted tissue as indicated in table 3.

Table 4:

risk for a systemic candidosis	transplanted tissue
28 - 42 %	liver
2 - 30 %	bone marrow
10 - 35 %	heart
0 - 20 %	kidney

The most frequently affected organs of cancer and immunocompromized patients are kidney, heart, intestines and lungs. Infections were more rarely observed in liver, spleen, central nervous system, lymph nodes, thyroid gland and larynx. In principle, each organ may be affected and the majority of patients suffer from simultaneous infections in more than one organ (up to four).

7. DIAGNOSIS

Antigen detection assays and *Candida* hemagglutination tests (C-HAT) are used most commonly in serological diagnosis of *Candida* infections. Since recently, *Candida* ELISAs (C-ELISA) has been commercially available.

The interpretation of the results in serology of *Candida* infections revealed to be very complex. On one hand, the widespread of transient colonization of healthy immunocompetent persons results in a significant anti-*Candida* antibody response and on the other hand, life-threatening systemic *Candida* mycoses in immunocompromized patients may not be accompanied by a significant increase in antibody titers. Therefore, the serological findings have to be interpreted with particular care. Systemic *Candida* infections do not reveal

specific symptoms thereby complicating the diagnosis of severe infections.

Yeasts show a complex composition of antigens which are recognized during superficial and/or severe invasive *Candida* mycoses by a variety of specific antibodies. The choice of the antigen preparation used in serological assays is of major importance for the outcome of the test results. By comparing several commercial antibody detection systems, significant discrepancies were observed. As a consequence, interpretation of serological findings becomes more complex but simultaneously offers a more differentiated view of the humoral immune response to this pathogen.

Serology of *Candida* infections is useful in

- (i): confirmation of a clinical finding
- (ii): monitoring of patients at high risk
- (iii): controlling and management of therapy in severe infections

The application of a single test system is generally not sufficient for the serological diagnosis of *Candida* infections. The combinations of different assays that differ in their antigen preparations are the current state-of-the art in serodiagnosis (2).

Antigen detection assays provide a rapid and reliable detection of the pathogen in blood samples during fungemia. Sensitivity and specificity of this method show large discrepancies: 19 % and 100 % and 51 % to 99 %. In patients with leukemia who are at high risk for acquiring severe *Candida* infections, sensitivity and specificity of antigen detection are 23 %-55 % and 66 %-100 % (3). In commercially available antigen detection systems no details are published about the composition of the detected antigen. Rheumatoid factors may generate false-positive results in up to 20 % of the cases. Therefore, the presence of RF-molecules must be taken into account in case of a positive result.

The *Candida* hemagglutination assay (C-HAT) is the most common test system in *Candida* serology. IgM antibodies are predominantly detected in this assay, but IgG antibodies in higher concentrations may also influence the titer. Therefore, a decrease of IgM antibody titers

induced by a successful anti-mycotic treatment may be masked by increasing concentrations of IgG antibodies resulting in constant C-HAT titer kinetics for a significant period of time. In these cases, no correlation of clinical findings and C-HAT results are then observed

Since recently, ELISA-test kits for the detection of Candida-specific antibodies are commercially available (C-ELISA). As mentioned above, the preparation and the composition of the antigen are of significant importance. On one side, purely cytoplasmatic antigen preparations are not sufficient for the use in highly sensitive and specific assays for the detection of the early colonization phase of the mycosis. On the other side, ELISA systems based on cell wall components are highly sensitive, because the humoral immune response is principally directed against outer surface structures of the pathogen. As a disadvantage, assays purely based on cell wall antigens cannot clearly discriminate between superficial infection and invasive mycosis.

Due to the detection of different types of antibodies, the HAT and ELISA do not correlate in detail (1, 2).

In the last few years, PCR was used for the detection of Candida albicans from different tissues and body fluids. In spite of first promising results, this technique is still limited to special demands and research laboratories due to its high technical expenditure.

Interpretation of serological results:

- IgM antibodies indicate an acute infection. A significant decrease of IgM-titers are detected up to several weeks post infection and after successful therapy. Elevated titers for an extended period of time are rarely observed.
- A high prevalence of IgG antibodies is observed in a healthy population due frequent or chronic infections.
- In case of infections of the mucosa, IgA antibodies are frequently detectable in parallel to rising IgG titers.

Table 5:

HAT	IgM-ELISA	IgG-ELISA	IgA-ELISA	interpretation
-	-	-	-	seronegative
-	-	+	-	acute infection unlikely infection in the past high seroprevalence of IgG antibodies
+	+	-	(+)	indication for an acute infection
+	+	±	(+)	in case of immunosuppression
+	+	+	(+)	additional antigen detection is recommended

8. DESCRIPTION OF THE TEST

SERION ELISA *classic* are indirect ELISAs (Enzyme-linked immunosorbent assays) for the detection of Candida-specific antibodies. An antigen preparation is coated to the solid support.

Due to different seroprevalence of anti Candida IgG- IgM- and IgA- antibodies in normal population, different predilutions of the serum samples (IgM 1:100; IgG, IgA: 1:1000) are necessary for optimum results.

Determination of all antibody classes can be performed quantitatively by a single point quantification method developed by Serion Immundiagnostica GmbH. Results are expressed as units per milliliter (U/ml). Quantitative results are obtained either by a lot specific standard curve and a calculation schedule supplied in the test kit or by the user friendly SERION *easybase* (order no. VT013) software.

A variety of antigens of Candida albicans have been described. The so-called mannan components and the 46 kd protein are the major antigens of this microorganism. Mannan consists of high molecular carbohydrate-protein complexes constituting the major component of the cell wall. The 46 kd protein is localized in the cytoplasm. Antibodies to the mannan components are observed in superficial infections as well as in healthy persons, whereas antibodies to the 46 kd protein are mainly detected in deep seated Candida infections.

The use of purely cytoplasmatic antigens for the serological monitoring of patients at high risk is not advisable (Repintigny 1989). A certain amount of mannan antigens seems to be essential for the detection of the colonization phase at an early stage of the infection. In contrast, cytoplasmatic antigens revealed (e.g. 46 kd enolase) to be important for the diagnosis of deep-seated infections (Zöller et al., 1991, Matthews et al., 1987, 1988, Walsh et al., 1991). A mannan reduced cellular extract with cytoplasmatic structures as well as cell wall components is used as the antigen for the SERION ELISA *classic*. Since both, intracellular and outer surface components are used, the detection of superficial and deep seated mycosis are possible.

According to recent studies, infections with non *Candida albicans* species (e.g. *glabrata* and *tropicalis*) can be detected with the SERION ELISA *classic* (personal communication, Dr W. Fegeler, Münster University).

9. STUDIES

9. 1. SERION-ELISA *classic* in comparison with the HAT

In a comparative study, the serum samples of 372 patients were analyzed with the SERION ELISA *classic* and a commercially available HAT. The IgG-ELISA correlated in 33.3 % with the HAT, the IgM-ELISA in 31.1 % and the IgA-ELISA in 26.7 % of all cases. A positive result in the ELISA assay correlated in every case with a positive or borderline value in the HAT. The number of borderline results was significantly lower in the ELISA than in the HAT indicating a better discrimination of positive and negative results in the ELISA.

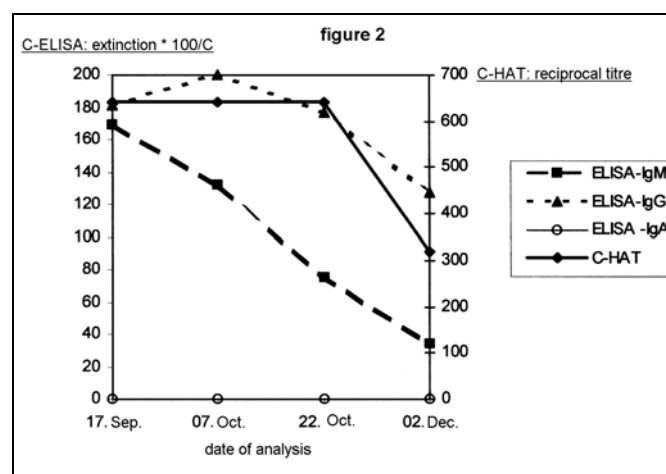
It is important to state that the ELISA and the HAT are two test systems that complement one another. Particularly when using a single serum sample or in monitoring patients at high risk, the differentiation of the immunoglobulin classes in the ELISA provides additional diagnostic information.

9.2. SERION ELISA *classic* and clinical findings

Analysis of a clinical case with the SERION ELISA *classic*

The benefit of the differentiation of the immunoglobulin classes can be seen from the course of a mycosis with constant HAT-titers (Figure 2, personal communication Dr W. Fegeler, Münster University). Whereas an interpretation according to HAT titers was not possible, the IgM titer kinetics as obtained by the ELISA showed good correlation with the clinical findings.

Diagram 2:



The 5-year-old male patient suffered from morbus down. Diagnostic findings revealed candida sepsis and infection with staphylococcus spec. The patient was treated with amphotericine B and 5-fluorocytosine every 4 weeks as well as subsequent application of fluconazole for therapy. At the end of therapy a complete recovery from Candida sepsis was observed.

Validity of cut off ranges in serodiagnosis of young children

Young children are at higher risk for deep-seated *Candida* infections due to a variety of basic diseases. Serodiagnosis by ELISA is very common in these cases. It is important to know that cut off ranges for most serological tests are well suited for the testing of adult patients, but do not necessarily reflect the situation in young children.

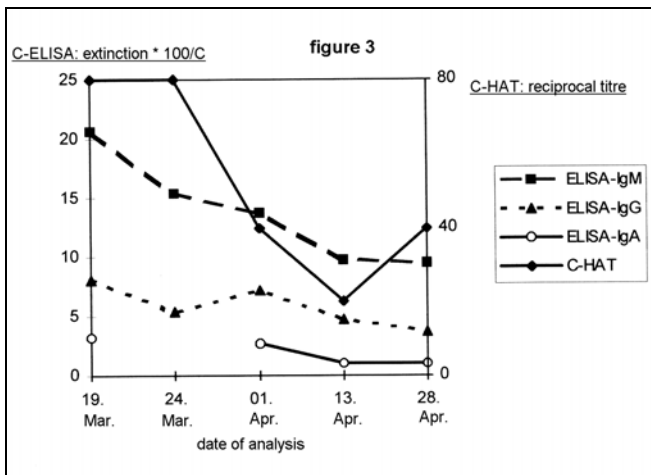
In the following study, 16 serum samples of healthy young children with an average age of 8 months were analyzed by the SERION ELISA *classic*. The results are summarized in table 5.

Table 6:

Ig-class	OD _{min}	OD _{max}	X	X+3SD	Cut off value for adults
IgM	0.02	0.25	0.09	0.20	0.20-0.28
IgG	0.001	0.22	0.07	0.17	0.47-0.84

Changes in antibody titers below the recommended cut off ranges are highly significant in serodiagnosis of young children up to 2 years. This fact was demonstrated by the course of disease of a 10 months old patient suffering from a systemic Candida infection as shown in Figure 3

Diagram 3:



The 10 months old patient suffered from a Candida endocarditis after surgery. The pathogen was isolated by culture from a biopsy taken from the heart. Therapy included the combined use of amphotericin B and 5-fluocytosine. No clinical findings were observed at the end of the treatment.

10. SUMMARY

- The complex antigen that consists of cytoplasmatic and an reduced amount of cell wall components provides the detection of anti-Candida antibodies in superficial and

deep-seated mycoses at different stages of the infection.

- In contrast to the HAT, the ELISA enables an immunoglobulin differentiated diagnosis providing the early detection of changes in antibody titer kinetics, which is of particular importance in therapy monitoring.
- The detection of antibodies to non *Candida albicans* species (*glabrata*, *tropicalis*) was observed by the SERION ELISA *classic*.

11. DIAGRAMS

Diagram 1: Serion ELISA *classic*: anti-Candida Antibody in normal population: Age distribution

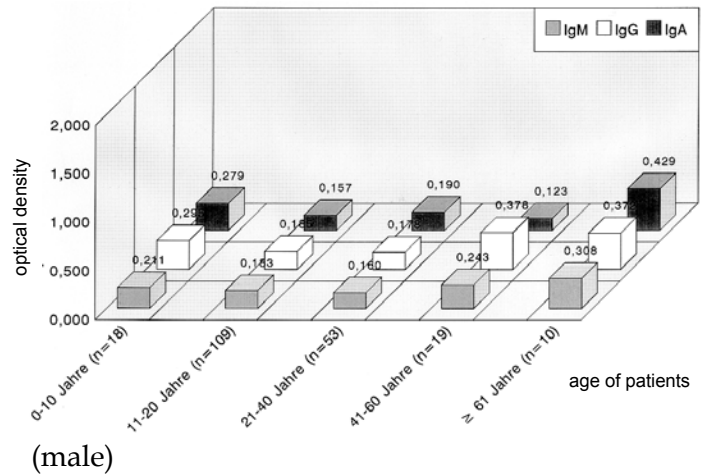


Diagram 2: Serion ELISA *classic*: anti-Candida Antibody in normal population: Age distribution (female)

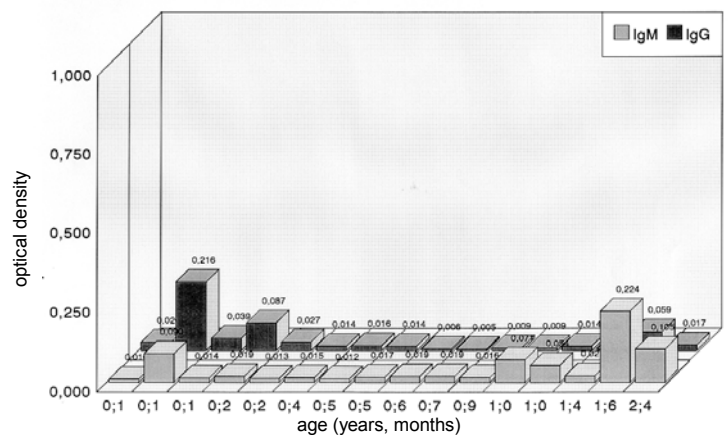


Diagram 3: Serion ELISA classic: anti-Candida Antibody in normal population: Age distribution (infants)

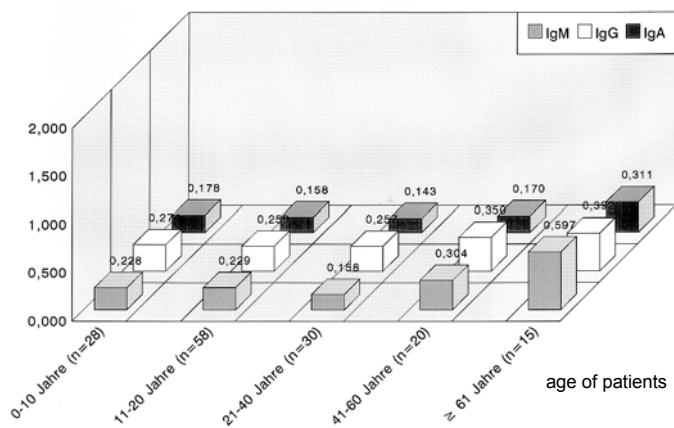
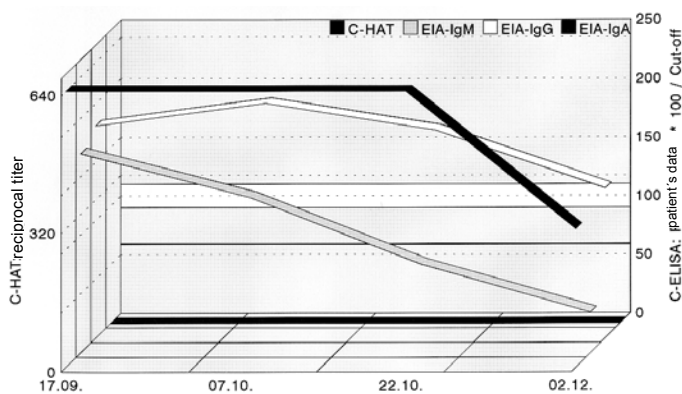


Diagram 4: Antibody kinetics of SERION ELISA classic candida albicans, with static Candida hemagglutination titer, under systemic antimycotic therapy.

patient male, 5 years old; Morbus down



Status at Candida infection of the skin as a secondary complication after staphylococcus infection.

Therapy: amphotericine B and 5-fluorocytosine iv (4 weeks). Subsequent oral therapy: fluconazole

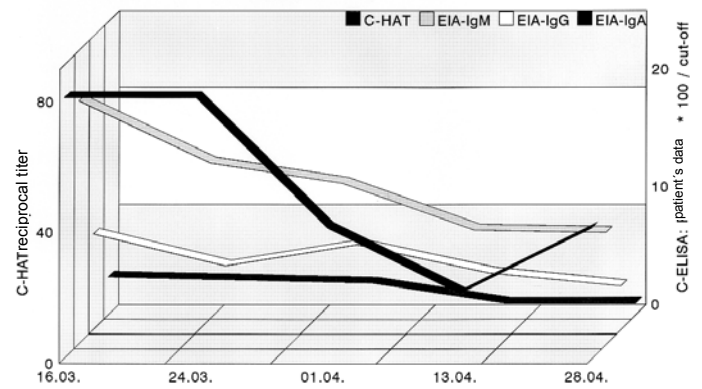
The IgM-Antibody titer correlates with the clinical findings.

Diagram 5:

Age-related evaluation of Antibody kinetics under systemic antimycotic therapy.

Female patient, 10 months; correction of congenital heart defect.

Candida endocarditis, verification by culture of candida from mitral valve.



Therapy: amphotericine B and 5-fluorocytosine iv (6 weeks); subsequent oral therapy: fluconazole

The IgM-Antibody titer correlates with the clinical findings. In this age group IgM titers >18,5 are discussed to be positive and < 8,5 negative

No clinical findings were observed at the end of the treatment.

12. BIBLIOGRAPHY

12.1 Possibilities of a differentiated Candida serology regime

(W. Fegeler, University of Münster (author) in: pilzdialog 4/1992, 61-62)
(translated by B. Gerstenecker, Serion Immundiagnostica GmbH, Germany)

In the past decade Candida serology plays a central role in the prevention and diagnosis of generalized mycoses.

The basis for this development was the widespread use of commercially available standardized test systems.

Indirect Candida hemagglutination assays (CHAT) are of particular importance. CHAT is commonly used as a "golden standard" for the evaluation of newly developed methods in Candida serology.

The more widespread mycological-serological diagnosis is applied, the more obvious the inherent limits and problems become. For example stagnating CHAT titers are problematic as no interpretation and no consequences in the therapy regime can be drawn.

The loss of the detection of titer dynamics, which is particularly featured by the CHAT is influenced by high Candida-specific IgG titers in patients with severe mycoses. Due to the competition specific IgG antibodies are now determining the CHAT titers. The half-life time of IgG antibodies with approximately 20 days is longer than of other immunoglobulin. As a consequence the CHAT titers decrease significantly slower. If a second explanation with the yeast occurs during this period a plateau of the CHAT titers results.

A solution of this problem may be an immunoglobulin-specific diagnosis.

For immunoglobulin-specific Candida serology, Candida ELISA (Enzyme-linked immunosorbent assay) was described by various authors in the middle of the 1980's (1,2). However these assays were not generally available and their use restricted to specialized laboratories. This lack is closed now by commercially available Candida-ELISA's.

Candida hemagglutination assay vs. Candida-ELISA

The limitations and application fields of the Candida ELISA were analyzed by comparison of an C-HAT (LD-Candida hemagglutination assay; Labor-Diagnostika, Heiden/Germany) and a Candida-ELISA (SERION ELISA *classic* Candida IgG/IgM/IgA; Serion Immundiagnostica GmbH, Würzburg / Germany)

Serum samples of 372 unselected patients out of routine testings were analyzed. 64.5% of the patients were at increased risk for the acquisition of severe mycoses (intensive care, hematological-oncological therapy).

Results

By quantitative interpretation 41 out of 372 serum samples (11,0%) showed positive titers ($\geq 1:320$) in CHAT (table 1). In Candida-ELISA IgM, IgG and IgA 17 sera (4,6%), 22 (5,9%) and 15 (4,0%), resp. revealed positive reactions. Only about 50% of CHAT were positive in Candida-ELISA.

In contrast to CHAT, Candida-ELISA proved to discriminate more clearly between positive and negative results. The amount of borderline results in Candida IgG-, IgM- and IgA-ELISA was 7,9%, 3,5% and 1,9%, respectively.

After pretreatment of the serum samples with anti-IgG antibodies (RF-absorbent; Behringwerke AG, Marburg/Germany) the number of equivocal results in CHAT decreased from 22,6% to 6,2%. The influence of Candida-specific IgG antibodies on the CHAT results is clearly documented.

Generally it is expected that predominantly IgM antibodies are detected by the CHAT. On this assumption a discrepancy exists between CHAT and Candida-ELISA: only 12 out of 41 sera which reacted positive in CHAT ($> 1:320$) showed positive results in the Candida-IgM-ELISA.

All sera reacting positive in CHAT ($\geq 1:640$) showed positive reactions with one immunoglobulin-class in Candida-ELISA. However, more IgM reactions were observed.

These results clearly indicate that both test systems partially detect different antibodies therefore rising the opportunity to a more sophisticated Candida serology schedule.

Identity of Candida-ELISA and CHAT results

Independent of the immunoglobulin-class detected in Candida-ELISA positive ELISA findings correspond with a probability of close to 100% with positive CHAT results.

Interpretation and application

Candida-ELISA offers a sophisticated laboratory diagnosis. Concerning therapy management Candida-ELISA reveals decisive profits.

Antigen composition directly influences the test results. It explains decreasing IgM reactions under therapy while CHAT titers still are positive or equivocal.

Due to the differences in antigen preparations the test results and their subsequent interpretation cannot be applied to similar test systems of different manufacturers.

Therefore the assay's manufacturer have to be regarded for the interpretation of the patient's data and the identification of the antibodies which were detected.

Summary

On the basis of the test results mentioned above the following conclusions have to be made:

- (i) CHAT and C-ELISA are not exchangeable but benefit from each other because different antibodies seem to be detected
- (ii) Positive Candida-ELISA are generally correlating with positive and equivocal results in Candida hemagglutination assay
- (iii) As a current working schedule CHAT is applied as screening test and due to the detection of IgM-, IgG- and IgA-antibodies Candida-ELISA offers the specification.

With the Candida-ELISA the potential of a differentiated Candida serology in laboratory

routine is considerably enlarged. It is our turn to explore and to use these diagnostic tools.

Bibliography

- Ansorg R, Kraus C:** Enzymeimmunoassay zum Nachweis von IgG- und IgM-Antikörpern gegen somatische Antigene von *Candida albicans*. Lab.med. 8, 396-399 (1984).
- Jones JM:** Laboratory Diagnosis of Invasive Candidiasis. Clin.Micobiol.Rev., 3, 32-45 (1990).
- Fegeler W:** Basisdiagnostik in der Candida-Serologie. pilzdialog 11-12 (1989)

Author's address:

W. Fegeler, MD
 Institute of Medical Microbiology
 of the Westf. Wilhelms-University
 Domagkstraße 10
 48149 Münster

Table A:

Results of 372 unselected serum samples
 -qualitative evaluation-

Candida-haemagglutination-Test (CHAT) (1)				
test	positive	negative	borderline	n
CHAT n	41	247	84	372
%	11.0	66.4	22.6	100
Candida-ELISA (2): different immunoglobulin-classes				
Ig	positive	negative	borderline	n
M n	17	342	13	372
%	4.6	91.9	3.5	100
G n	22	323	27	372
%	5.9	86.8	7.3	100
A n	15	350	7	372
%	4.0	94.1	1.9	100

(1) LD-Candida-haemagglutination test, Labor Diagnostica, Heiden, Germany

(2) SERION ELISA classic Candida, Serion Immundiagnostica GmbH, Würzburg/Germany

12.2 Verification of differentiated Candida serology

(Tietz H.-J, Tausch I. (authors; in: pilzdialog 4/1993, page 55-56)
(translated by B. Gerstenecker, Serion Immundiagnostica GmbH, Germany)

Pro and contra - discussion about Candida serology is still pending. The hemagglutination assay (HAT) is easy to handle and widespread in use. As a universally applicable assay it allows a summary point of view of the antibody status. One recognizes if an immunogenic systemic explanation with Candida antigen took place or not. Whereas short-termed significant titer-increases indicate actuality, spontaneous reciprocal courses indicate deficiencies in immune response, which are of negative prognostic outcome if paralleled with simultaneous antigenemia. Now, potential of interpretation of undifferentiated antibody determination ends. The demand of the clinicians to get indication of the severeness on the sickness by the immune system signalling a systemic invasion of the pathogen is not provided.

Is there the demand for differentiated Candida serology besides a rough orientating laboratory diagnosis? The tools are still available (1). In this examinations the class-specific enzyme immunoassay of SERION, Würzburg/Germany (SERION ELISA *classic* Candida IgM, IgG, and IgA) are evaluated.

Patient's sera

For test evaluation patient's paired sera were selected for high reactivity in an indirect hemagglutination assay (distributor: LD Labordiagnostika, Heiden/Germany). Beside the criterion "sero-reactive" (3-fold titer increases and/or serum titer $\geq 1:640$) the number of serum samples per patient should allow to monitor the time course qualitatively. All selected kinetics (n=91, i.e. 46% out of all patients staying longer than 1 week in an intensive care unit) stem from intensive care medicine. 401 out of 818 sera which were pre-tested in HAT were used for differentiated Candida serology (IgM, IgG, IgA). The resulting patients were grouped as follows: HAT titer 1:40 to 1:80 (n=31), 1:80 to 1:320 (n=172), $\geq 1:640$ (n=198).

Results

Examination of the antibody response was part of the mycological routine monitoring. Spectrum of pathogens in intensive care medicine was dominated by Candida albicans (45 patients), C. glabrata (9 patients) and C. tropicalis (1 patient). Mixed infections (19 patients) were caused predominantly by combinations of C. albicans and C. glabrata (11 patients). C. krusei appeared in only two cases as accompanied yeast flora. In figure 1 dynamics of antibody titers of patient groups at high risk are shown. Within a few days the basic titers of approx. 1:40 increased 32-fold to $\geq 1:1280$.

After approval of significance and selection of the test sera the class of the inducing antibodies were examined. In table 1 the paired sera were differentiated into immunoglobulin classes. 71 out of 91 patients developed a IgM response which is regarded as a characteristic feature of a primary infection. In 31 patients solely IgM antibodies were detected; in 40 cases combinations of IgG and IgA appeared.

Due to the significance of the time courses and the specificity of the IgM antibodies, intensive care medicine is characterized as a focus of an early mycogenous burden. Discrepancies of a positive HAT reaction and immunoglobulin-class independent negative ELISA results in 5 patients seem to be caused by the presence of immune complexes. Therefore not any HAT titer is correlated with the presence of antibodies! On the other side any antibody constellation detected in ELISA was accompanied by positive HAT findings. Due to the reliability the HAT should be applied prior to the immunoglobulin-class specific antibody analysis.

Surprisingly IgG antibodies were detected in parallel with IgM antibodies. This finding may indicate a latent Candida antibody "body building" prior to admittance of the patient to the hospital.

Dominant solely IgG antibody courses in female patients seem not to be caused by chance as vaginal colonization with C. albicans is accompanied.

Both the rapid IgG seroconversion and the generation and persistence of IgG antibodies, which follows the disappearance of IgM

antibodies, could protectively influence the course of the infection. Preliminary clinical observations support this theory.

As a result of the described results *C. glabrata* seems to face a rather naive immune system as exclusively IgM responses were observed. Only 8 of all patients tested showed the appearance of a complete arsenal of IgM- IgA- and IgG antibodies. In table 2 titer dynamics during seroconversion are shown. 36 patients showed no increases of the specific immune reaction in comparison to the initial values (findings). Consolidation of high antigen titers of 1:16 to titers \leq 1:4 were observed in 35 cases. However, 8- to 16-fold regressions of antigen titers are regarded as predominantly influenced by the therapy (2). Infrequently, an escalation of the mycological findings were observed, which ended lethally in spite of the therapy. Beside a lack of IgG antibodies the pathognomonic discrepancy between "early" and "late" antigen titers are obvious.

Conclusions

The respect on *Candida albicans* which currently is one of the most important pathogens demand a high level of information during the management of patients at risk. A sophisticatedly established differentiated antibody status (increase, persistence, regression appearance of any immunoglobulin class) is not only important for a better understanding of the host's immune situation. The assistance of immunoglobulin patterns could be beneficial for the planning of therapy regimes and the interpretation of the prognostic outcome. The current available methods (HAT and enzyme immunoassays) proved to be a rational system of user-friendly screening and confirmation test.

Bibliography:

Fegeler W: Möglichkeiten einer differenzierten *Candida*-Serologie. *pilzdialog* 4/1992, 61-62

Tausch I, Tietz H-J, Meyer R, Klug C, Staffa G: Therapie systemischer Mykosen mit Fluconazol. *mycoses* (1991) in press.

Authors address:

Hans-Jürgen Tietz, MD
Dermatological clinic and polyclinic
of the Charité
Schumannstraße 20/21
10117 Berlin

Table 1:

Differential *Candida* serology: IgM-, IgA- and IgG courses of 91 intensive care patients who showed HAT reactivity

Combination	IgM	IgG	IgA	IgM IgG	IgM IgA	IgG IgA	IgM IgG IgA	neg
Number of courses	31	11	2	28	4	2	8	5

Table 2:

***Candida* antigen titers (Ramco) in patients with significant antibody status**

Titer in titration steps	-4	-3	-2	-1	0	+1	+2	+3	+4	+5
Number of courses	3	11	21	16	25	4	4	1	0	1

12.3 Annex

1. L. de Repentigny, 1989: "Serological Techniques for Diagnosis of Fungal Infection", *Eur. J. Clin. Microbiol. Infect. Dis.*, Vol 8, No. 4, p. 362-375
2. F. Meunier, 1989: "Candidiasis", *Eur. J. Clin. Microbiol. Infect. Dis.*, Vol 8, No. 5, p. 438-447
3. L. Zöller et al., 1991: "Enzyme Immunoassays for Invasive *Candida* Infections: Reactivity of Somatic Antigens of *Candida albicans*", *J. of Clin. Mic.*, Vol. 29, No. 9, p. 1860-1867