An Automated Enzymatic Method for Measurement of D-Arabinitol, a Metabolite of Pathogenic *Candida* Species

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An automated enzymatic method was developed for the measurement of D-arabinitol in human serum. The assay is based on a novel, highly specific D-arabinitol dehydrogenase from *Candida tropicalis*. This enzyme catalyzes the oxidation of D-arabinitol to D-ribulose and the concomitant reduction of NAD⁺ to NADH. The NADH produced is used in a second reaction to reduce *p*-iodonitrotetrazolium violet (INT) to INT-formazan, which is measured spectrophotometrically. The entire reaction sequence can be performed automatically on a COBAS MIRA-S clinical chemistry analyzer (Roche Diagnostic Systems, Inc., Montclair, N.J.). Replicate analyses of human sera supplemented with D-arabinitol over a concentration range of 0 to 40 μ M demonstrated that the pentitol could be measured with an accuracy of $\pm 7\%$ and a precision (standard deviation) of $\pm 0.4 \mu$ M. Serum D-arabinitol measurements correlated with those determined by gas chromatography ($r = 0.94$). The enzymatic method is unaffected by L-arabinitol, D-mannitol, or other polyols commonly found in human serum. Any of 17 therapeutic drugs potentially present in serum did not significantly influence assay performance. Data illustrating the application of the assay in patients for possible diagnosis of invasive candidiasis and the monitoring of therapeutic intervention are presented. The automated assay described here was developed to facilitate the investigation of D-arabinitol as a serum marker for invasive *Candida* infections.

Candida infections are difficult to diagnose (3, 6). These infections are a source of significant morbidity and mortality in hospitalized patients (5, 8). Arabinitol is a pentitol capable of existing in two forms called enantiomers or stereoisomers that are nonsuperimposable mirror images of each other. Most medically important species of *Candida* including *C. albicans*, *C. tropicalis*, and *C. parapsilosis* produce measurable amounts of the D enantiomer of arabinitol in vitro (1, 7, 17). In contrast, known vertebrate metabolic pathways produce only the L enantiomer of arabinitol (21). There is considerable evidence, moreover, that patients with invasive candidiasis have higher serum D-arabinitol (DA) levels (2, 7, 18, 24, 25) and higher serum DA/creatinine ratios (2, 24, 25) than uninfected control patients. Given these observations, DA has been recognized as a potentially useful diagnostic marker for invasive candidiasis. Its routine use in clinical laboratories, however, has been limited by the available measurement techniques.

Enantioselective measurements of DA in human serum were originally made by combined microbiologic-gas chromatographic (GC) (2) and enzymatic-GC techniques (24). These methods are relatively slow, allowing for only a few assays to be performed each day. Recently, two newer GC methods that use columns with a chiral stationary phase capable of separating enantiomers of arabinitol have been developed (16, 25). Although these methods are highly specific for DA and do not require serum pretreatment by enzymatic or microbiologic techniques, they are generally too technically demanding and cumbersome for routine use

in clinical laboratories. A more practical enzymatic fluorometric method was developed by using a *Klebsiella pneumoniae* D-arabinitol dehydrogenase (DADH; EC 1.1.1.11) (18, 19). However, the cross-reactivity of this dehydrogenase with D-mannitol, which is normally present at various concentrations in human serum, significantly reduces the specificity of this assay.

In this report, we describe an enzymatic chromogenic assay for DA in human serum. The assay is based on the following reaction sequence:

where DADH is the enzyme D-arabinitol dehydrogenase from *C. tropicalis* and INT is *p*-iodonitrotetrazolium violet.

In this scheme, the amount of INT-formazan produced is proportional to the DA content of the sample. The concentration of INT-formazan is determined spectrophotometrically at 500 nm. Because of the specificity of the DADH from *C. tropicalis* (14), this method avoids the cross-reactivities of previous enzymatic DA assays (18, 19). The reaction steps have been adapted so that they can be performed automatically on a COBAS MIRA-S clinical chemistry analyzer, thereby simplifying the procedure. The high degree of specificity of the assay, coupled with the automated method, should make it suitable for routine use in clinical laboratories.

MATERIALS AND METHODS

DA assay reagents. Three reagents and one diluent were used in the automated assay for DA. The DADH-NAD⁺

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reagent contained 1.5 U of purified *C. tropicalis* DADH per ml, 2.23 mM NAD⁺ (catalog no. N-1636; Sigma Chemical Co., St. Louis, Mo.), 30 µg of bovine serum albumin per ml, 10 mM MgSO₄, 100 mM NaCl, and 10 mM Tris-acetate (pH 6.0). The DADH enzyme was purified from *C. tropicalis* (14) and has been cloned and expressed in *Escherichia coli* (11). The specific activity of the enzyme was approximately 150 U/mg, where 1 U is the amount of enzyme necessary to produce 1 µmol of NADH per min at 25°C in a buffered assay mixture containing 50 mM DA and 1.5 mM NAD⁺ (14). The DADH-NAD⁺ reagent was prepared fresh each day and was stored at 4°C prior to use. No significant loss of DADH activity was observed upon storage of this reagent at 10°C for 4 h. Stock solutions of DADH (10 mg/ml) were stable for at least 6 months when they were stored at -70°C in 0.1 M sodium phosphate (pH 7.0)-0.2 M NaCl-5 mM MgSO₄.

The coupling reagent consisted of a filtered solution (pore size, 0.2 µm) containing 3.18 mM INT (catalog no. I-8377; Sigma), 66.6 mM EDTA (pH 9.0), and 0.03% pluronic 25R2 (BASF). The latter is a defoaming agent (10). The coupling reagent was prepared fresh each month and was stored in the dark at 4°C. Under these conditions, the reagent was stable for at least 3 months.

The diaphorase reagent contained 60 U of *Clostridium kluyveri* diaphorase (EC 1.8.1.4) (catalog no. D-2381; Sigma) per ml in phosphate-buffered saline. Stock solutions were prepared twice a year and were stored at -70°C in 0.2-ml aliquots. Aliquots were subjected to no more than two freeze-thaw cycles prior to use.

The diluent was 1.0 M sodium glycine (pH 10.5). When this solution was combined with the sample and the DADH-NAD⁺ reagent, the pH was reduced to 10.0, the optimum for DADH catalysis.

Automated enzymatic assay of DA. Serum samples were diluted 1:2 (vol/vol) with 10 mM sodium citrate (pH 4.0), boiled for 10 min, cooled on ice, and then centrifuged at 10,000 × g for 10 min. The extent of sample dilution used was the minimum that yielded a precipitate during the boiling step that was pelletable by centrifugation at 10,000 × g. This pretreatment eliminated endogenous enzymatic activities capable of producing NADH. The supernatants were removed and analyzed for DA or were stored at -20°C for later analysis. Storage of serum samples that had been frozen prior to or subsequent to this pretreatment step did not affect the assay results.

A two-stage enzymatic assay protocol is performed automatically on the COBAS MIRA-S analyzer (Roche Diagnostic Systems, Inc., Montclair, N.J.). The COBAS systems are a series of autoanalyzers commonly found in hospital chemistry laboratories and are used in a wide variety of commercial tests for proteins, hormones, metabolites, ions, and therapeutic and abused drugs. The COBAS MIRA-S analyzer is distinguished by its ability to maintain reagents at subambient temperature. This instrument also has the flexibility of handling up to three reagents and is equipped with a sensitive spectrophotometer capable of accurately measuring absorbance changes of as low as 0.0001. Functions are performed by the instrument in increments, or cycles, of 25 s. For the DA assay, the instrument carousel is maintained at 32°C while the reagents are stored on the instrument at 10°C. By using a cycle time of 25 s, the following sequence of steps is carried out. (i) During cycle 1, the instrument mixes 85 µl of pretreated sample, 10 µl diluent, and 100 µl DADH-NAD⁺ reagent. (ii) Incubation occurs in cycles 2 to 36. (iii) During cycle 37, the instrument adds 15 µl of coupling reagent and then 5 µl of distilled water. (iv) During cycle 38,

the instrument records the A_{500} of the reaction mixture. (v) During cycle 39, the instrument adds 3 µl of diaphorase reagent and then 5 µl of distilled water. (vi) During cycle 40, the instrument records the A_{500} . During cycles 1 through 36 (15 min), the DADH-catalyzed conversion of sample DA and NAD⁺ to D-ribulose and NADH proceeds to about 75% completion. The distilled water additions at cycles 37 and 39 are required to rinse the pipette tip. The absorbance measurement at cycle 38 provides the background absorbance of the sample. During cycle 39, the NADH formed during the initial stage is quantitatively oxidized by INT to give INT-formazan. The assay result is recorded as the difference in absorbance measured at cycles 40 and 38. This is converted to a DA concentration (in micromolar) through the use of a calibration curve.

Unless specified otherwise, samples were assayed in duplicate and the mean result is reported. The instrument performs tests in a staggered, overlapping manner by starting assays while the initial incubation stage (cycles 1 to 36) of previous assays is in progress. As a result, maximum throughput is 36 assays per h.

Assay calibration. Assay calibration curves were generated daily. For this purpose, six serum calibrators were prepared by supplementing a normal human serum pool containing an endogenous DA concentration of approximately 1.0 µM, determined as described below, with DA in 5-µM increments to yield concentrations of 1 to 26 µM. The calibration samples were analyzed in duplicate. Sample DA concentrations were determined by reference to the linear least-squares fit to the calibration values.

Determination of endogenous serum DA concentration. A modification of the procedure of Wong and Brauer (24) was used to determine the endogenous concentration of DA in the calibration serum. The calibration serum was initially depleted of DA enzymatically by the addition of DADH and NAD⁺. The NADH that was formed was converted back to NAD⁺ by lactate dehydrogenase and pyruvate. Incubations contained 0.5 ml of calibration serum supplemented with 0 or 20 µM DA, 4 µl of 250 U of *C. tropicalis* DADH per ml, 10 µl of 1,000 U of lactate dehydrogenase per ml, 10 µl of 125 mg of sodium pyruvate per ml, and 10 µl of 50 mM NAD⁺. After incubation at 30°C for 2 h, the incubation mixture was pretreated with heat and was assayed as described above. Endogenous DA could be calculated from the assay response obtained when the sample was (R_{+0}) or was not (R_{-0}) subjected to enzymatic depletion and when the same sample supplemented with 20 µM DA was (R_{+20}) or was not (R_{-20}) subjected to the enzymatic depletion. If it is assumed that the enzyme pretreatment depletes a fixed fraction of the endogenous DA regardless of the initial amount present, the background signal (b) not associated with DA is then

$$b = \frac{(R_{-0} R_{+20}) - (R_{+0} R_{-20})}{(R_{-0} + R_{+20}) - (R_{+0} + R_{-20})}$$

The assay response that was due to endogenous DA alone is $x = R_{-0} - b$, and the endogenous concentration of DA is x divided by the initial slope of the assay calibration curve.

Approximately 85% of b , which was usually about 0.004, was due to the absorbance of the diaphorase reagent. The remainder was attributable to the nonenzymatic interaction of INT with a component(s) of the boiled serum.

Clinical evaluation. A standard calibration curve was established each morning before analyzing coded serum samples from prospectively monitored patients. Each sample was analyzed for DA and creatinine on the COBAS MIRA-S

FIG. 1. Calibration curve for the DA assay. A normal human serum pool containing $0.92 \mu\text{M}$ endogenous DA was supplemented with various amounts of DA and assayed in duplicate. The x axis gives the total concentration of endogenous and added DA. ΔA_{500} values (changes in the A_{500}) are in thousandths (i.e., $\times 10^{-3}$).

analyzer. In the present study, the calculated DA/creatinine ratio is presented with units of micromolar per milligram per deciliter. In contrast, previous investigators calculated this ratio so that it had no units (2, 24, 25). A DA/creatinine ratio of $\geq 4 \mu\text{M}/\text{mg}/\text{dl}$, which corresponds to the mean plus 3 standard deviation of the ratio measured in sera from 400 healthy volunteers (22), was considered to signify a high likelihood of invasive candidiasis. Serum DA/creatinine ratios were compared with the effect of antifungal therapy and the incidence of fungemia as detected by means of lysis centrifugation blood culture (Isolator; DuPont, Wilmington, Del.).

Other materials. DA, L-arabinitol, D-mannitol, D-sorbitol, ribitol (adonitol), galactitol (dulcitol), and xylitol were obtained from Aldrich Chemical Co. (Milwaukee, Wis.). Erythritol, threitol, D-glucose, D-mannose, D-galactose, D-fructose, bovine serum albumin, and sodium pyruvate were from Sigma. Unless indicated otherwise, the therapeutic drugs used in the present study were obtained from Sigma. Fluconazole was from Pfizer, Inc. (New York, N.Y.), gentamicin was from Schering-Plough Corp. (Madison, N.J.), vancomycin was from Eli Lilly (Indianapolis, Ind.), and methylprednisone was from Upjohn Co. (Kalamazoo, Mich.).

RESULTS

Calibration curve. Figure 1 shows a typical DA calibration curve. In replicate experiments on 15 different days, linear calibration curves were obtained as $y = (3.86 \pm 0.48) +$

TABLE 1. Recovery of DA in assays of serum specimens from 12 healthy humans

DA concn added (μM)	Observed DA concn (μM) ^a		% Recovery ^b
	Unsupplemented sample	Supplemented sample	
3	1.8 ± 0.3	5.0 ± 0.4	107
4	0.1 ± 0.1	3.9 ± 0.5	95
6	0.5 ± 0.3	6.4 ± 0.1	98
6	0.3 ± 0.1	6.4 ± 0.3	102
8	0.3 ± 0.3	8.6 ± 0.4	104
11	1.2 ± 0.3	13.0 ± 0.3	107
11	0.3 ± 0.2	11.8 ± 0.2	105
16	0.3 ± 0.3	16.4 ± 0.1	101
16	0.5 ± 0.2	17.1 ± 0.5	104
20	2.3 ± 0.4	22.9 ± 0.3	103
40	0.8 ± 0.2	41.2 ± 0.5	101
40	0.8 ± 0.4	41.7 ± 0.7	102

^a Measurements taken from the standard curve are means of quadruplicate analyses.

^b Difference in observed DA concentration between supplemented and unsupplemented samples divided by DA concentration added.

$(1.160 \pm 0.078)x$, with $r = 0.999 \pm 0.001$. In these experiments, stock solutions of DADH were stored at -70°C and were subjected to up to five freeze-thaw cycles prior to use. The high day-to-day variability (coefficient of variation = 6.7%) observed in the slope may have been caused by this repeated freezing-thawing of the DADH enzyme, which resulted in a slight (5 to 10%) reduction in enzymatic activity.

Precision and accuracy of the DA assay. The precision of the DA assay was assessed through replicate assays of a pool of normal human serum containing $1.0 \mu\text{M}$ endogenous DA and supplemented with various amounts of DA. When 0, 3, 7, 13, and $21 \mu\text{M}$ DA was added, 0.73 ± 0.31 , 4.01 ± 0.47 , 7.94 ± 0.44 , 14.2 ± 0.4 , and $22.0 \pm 0.4 \mu\text{M}$ DA, respectively, was observed (observed values are means $\pm$ standard deviations of 18 replicate analyses). The standard deviation of the assay averaged $0.4 \mu\text{M}$ DA and was unrelated to the DA concentration.

The recovery of DA was determined by supplementing individual serum samples with various amounts of DA (Table 1). Recoveries are based on the ratio of the measured DA concentration in the supplemented sample and the sum of the measured concentration in the unsupplemented sample plus the supplemented amount. The average recovery was $102\% \pm 3\%$. The mean endogenous DA concentration in the 12 serum samples from healthy adult subjects was $0.8 \pm 0.6 \mu\text{M}$.

To determine the correlation of the present enzymatic method with two-dimensional GC (25), 90 serum samples were obtained from 46 patients with a wide variety of fatal illnesses. The samples were drawn not more than 5 days preceding the date of the patient's death. Because DA clearance is by glomerular filtration and 39 of the 90 samples were from individuals with significant renal impairment (creatinine concentration, $\geq 2.0 \text{ mg}/\text{dl}$), elevated levels of DA were encountered frequently, even though none of the patients had blood cultures positive for *Candida* spp. or histological evidence of invasive candidiasis at autopsy. The samples were analyzed for DA by the enzymatic method and two-dimensional GC (25). As illustrated in Fig. 2, the correlation (r) of the two methods was 0.94, with a slope of 0.97.

Specificity and interfering substances. The assay response

FIG. 2. Correlation between DA concentrations in patient sera determined by GC and the automated enzymatic method ($r = 0.94$). A pool of serum from healthy humans containing 1.5 μM endogenous DA was used for calibration of the enzymatic method.

was assessed with a series of sugars and sugar alcohols added to human sera at concentrations at least 10-fold greater than the highest concentration reported in sera from healthy humans (9, 15). Table 2 shows that only xylitol and galactitol produced a measurable response, and these were 3.1 and 1.2%, respectively, of the response observed with an equimolar concentration of DA.

TABLE 2. Specificity of the DA assay^a

Compound added	Concn (μM)	Apparent DA concn (μM)
D-Arabinitol	50	50
L-Arabinitol	50	0
Ribitol	50	0
Xylitol	50	1.55
D-Sorbitol	50	0
D-Mannitol	120	0
Erythritol	50	0
Threitol	50	0
Galactitol	50	0.60
D-Fructose	500	0
D-Galactose	500	0
D-Mannose	750	0
D-Glucose	5,000	0

^a Assays were of a pool of serum from healthy humans supplemented with the polyols and sugars listed in the table at the indicated concentrations. The apparent DA concentration was calculated as the concentration of DA determined for the supplemented serum pool minus the endogenous DA concentration of the pool.

The effect on the assay of 17 therapeutic drugs at concentrations 10-fold greater than the highest in vivo concentration reported following therapy was tested (13). The following drugs were added to the serum sample prior to the boiling step at the indicated test concentration (in micrograms per milliliter, unless indicated otherwise): azathioprine, 10; methylprednisone, 120; prednisolone, 12; prednisone, 12; amphotericin B, 20; fluconazole, 81; ketoconazole, 70; chloramphenicol, 250; cefaclor, 230; ciprofloxacin, 43; erythromycin, 200; gentamicin, 120; piperacillin, 8; sulfamethoxazole, 400; trimethoprim, 20; vancomycin, 630; and heparin, 8 U/ml. None of the drugs was found to affect assay response in the presence of 0 or 20 μM added DA.

Patient studies. Figures 3a and b illustrate the time course of serum DA and DA/creatinine ratio determinations in the sera of two granulocytopenic patients with disseminated candidiasis. Blood cultures were performed on each day for which datum points are shown in Fig. 3. A DA/creatinine ratio of $>4 \mu\text{M}/\text{mg}/\text{dl}$ was detected earlier than fungemia in both patients. The time course depicted in Fig. 3a shows a progressive increase in the DA/creatinine ratio to $>9 \mu\text{M}/\text{mg}/\text{dl}$ following the initiation of amphotericin B therapy. Results of in vivo studies with a rabbit model system suggest that this is caused by further progression of the invasive disease, which declines only slowly in response to therapy (23). The eventual decline in the DA concentration and the DA/creatinine ratio (Fig. 3a) coincided with improvement of the patient during the course of amphotericin B therapy. The *Candida* infection in the patient whose results are presented in Fig. 3a was eventually resolved. The time course depicted in Fig. 3b shows a much greater increase in the DA/creatinine ratio for the patient (to $>18 \mu\text{M}/\text{mg}/\text{dl}$) compared with that for the patient in whose results are depicted in Fig. 3a. The persistent elevation of the DA concentration and the DA/creatinine ratio coincided with failure of antifungal therapy in this patient; death occurred on day 20.

DISCUSSION

The enzymatic DA assay described here avoids many of the problems associated with previous GC techniques for the measurement of DA in human serum (2, 16, 24, 25). In the combined microbiologic-GC technique of Bernard et al. (2), DA concentrations are calculated as the difference between serum arabinitol levels determined by GC before and after sample incubation with a strain of *C. tropicalis* which consumes DA once the preferred carbon sources are exhausted. This method requires a 24-h incubation step and is susceptible to interference by antifungal drugs. Its limit of detection of DA in serum is 0.7 μM (2). Alternatively, DADH from *K. pneumoniae* could be used for the removal of DA from serum. In this approach, DA levels are calculated as the difference between arabinitol levels determined by GC in untreated and enzyme-treated sera (24). This combined enzymatic-GC method is unaffected by antifungal drugs and can be completed within a few hours. Each specimen tested by this method, however, must be analyzed twice by GC to determine the concentration of DA. In the GC-mass spectrometry technique of Roboz et al. (16), enantioselective isolation of DA from derivatized specimens is accomplished by chromatography on a single GC column containing a chiral cyclodextrin-based stationary phase. In general, these columns have a limited lifetime, making the procedure expensive and time-consuming. The two-dimensional GC technique of Wong and Castellanos (25) is both sensitive and highly specific for DA. In this technique, each

FIG. 3. Time course of serum DA level and DA/creatinine ratio determinations in granulocytopenic patients with disseminated candidiasis. Fungemia was detected on days 63 (a) and 13 (b) by lysis centrifugation blood culture (BC) on blood drawn on day 60 (a) and day 11 (b), respectively.

specimen is fractionated successively over GC columns containing a conventional and a chiral stationary phase. Samples assayed by this technique were used in the present study as a standard of comparison (Fig. 2).

Figure 2 shows the correlation between the present enzymatic method and the standard GC method. The source of the apparent higher level of discrepancy between the two methods at high DA concentrations is unknown. The enzymatic method shows recovery of DA similar to that by the GC method. Possibly, the deviations are associated with the standard deviations observed with the GC method, which are approximately twice those of the enzymatic method (24).

Two other basic assay approaches were considered before the enzymatic format described in this report was chosen. A more sensitive fluorogenic method for the direct detection of NADH was rejected because relatively few automated clinical chemistry analyzers are equipped with fluorimeters. A colorimetric assay similar to the enzymatic method but that used a concerted, one-step reaction scheme would have allowed a kinetic measurement rather than an endpoint measurement of the DADH reaction. A one-step reaction scheme would avoid the possibility of end product inhibition of the DADH reaction at high DA concentrations. However, the method introduced errors from the nonenzymatic reduction of the coupling dye, INT, by components of the boiled serum. When INT was included (224 μ M) in the initial incubation stage, its nonenzymatic reduction yielded a change in the A_{500}/min of 0.008, which is roughly equivalent to 5 μ M DA. Because of variations among serum specimens, this large background signal led to unacceptable variations in the measured DA levels. In the assay protocol as currently configured, inclusion of 4.5 mM EDTA in the second incubation stage reduces the rate of nonenzymatic A_{500} generation from INT by eightfold. Inclusion of EDTA in a one-step procedure is precluded by the dependence of DADH on Mg^{2+} .

Attempts to eliminate the manual sample pretreatment step were unsuccessful. The boiling step was necessary to eliminate any contaminating NAD^+ reductases present in

the serum sample. In limited studies, deproteinization of the serum by ultrafiltration provided equivalent results, but it was no less laborious. The enzymatic assay, as configured in the present study, is limited by the need for very precise instrumentation. The spectrophotometer must be capable of accurately measuring changes in absorbance as low as 0.0001 and handling three reagents stored under refrigerated conditions. Fortunately, the COBAS MIRA-S analyzer was able to accommodate these requirements.

An important positive feature of the enzymatic method is its low cross-reactivity with other polyols usually found in human serum. Among the polyols tested as potential substrates in the assay, slight reactivity relative to that observed with DA was found with xylitol (3.3%). Since xylitol is present in serum from healthy humans at a fivefold lower concentration than DA (15, 24), the xylitol cross-reactivity would be expected to result in a less than 1% overestimation of the DA concentration even at basal serum DA levels determined by the technique described here. By contrast, the enzymatic fluorogenic assay for DA developed by Soyama and Ono (18, 19) uses partially purified *K. pneumoniae* DADH, which can utilize D-mannitol as a substrate (4, 12). Since the rate of this reaction is 20% that of DA and the concentration of D-mannitol in serum is variable and is, on average, about 50% greater than that of DA (9, 24), the measured serum DA concentrations can be affected in some samples. In addition to the cross-reactivity of D-mannitol, the possible presence of contaminant NAD^+ oxidoreductases in the relatively crude DADH preparation may account for the elevated DA concentration (6.5 μ M) in serum from healthy human subjects found by those investigators relative to that found in the present study (mean, 0.8 μ M; $n = 12$) and by other investigators (20, 24, 25).

The enzymatic method described here combines the advantages of relative simplicity, high specificity, and analytical sensitivity. The method has been found to be useful for a study of experimental disseminated candidiasis in rabbits, in which it was shown that serum DA levels correlate with concentrations of *C. albicans* in tissue and can be reduced in

response to antifungal therapy (23). DA time course results in humans (Fig. 3a and b) suggest that the method, in conjunction with other clinical indications, may provide earlier detection of disseminated candidiasis than is currently possible by blood culture and may potentially facilitate the earlier administration of therapy. An ongoing clinical trial investigating the potential role of monitoring the serum DA concentration by the present enzymatic assay has enrolled more than 200 patients and analyzed over 3,000 serum samples (22). The results of that study thus far demonstrate that rapid detection of DA by the enzymatic assay in serially collected serum samples permits detection of invasive candidiasis in hospitalized patients, the early recognition of fungemia caused by *C. albicans* and *C. tropicalis*, and potential therapeutic monitoring in DA-positive patients. It is anticipated that this new, facile enzymatic technique for the measurement of DA will ultimately lead to improved diagnosis and therapeutic monitoring of invasive candidiasis.

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