Original Article

Study on the Influence of Pretreatment Temperature for Plasma (1→3)- β -D-Glucan Measurement Using Kinetic Turbidimetric *Limulus* Assay and Clinical Evaluation of (1→3)- β -D-Glucan Measurement Methods

Takeshi Mori, Makiko Matsumura

Division of Haematology, Department of Internal Medicine, Juntendo University School of Medicine.

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Abstract

We clinically evaluated three commercially available (1→3)- β -D-glucan measurement methods for the diagnosis of fungal infections: the kinetic turbidimetric, the end-point chromogenic, and the kinetic chromogenic *Limulus* tests. For the kinetic turbidimetric assay, we examined a dilution and heating pretreatment method at 70°C for plasma to eradicate irregular (1→3)- β -D-glucan values caused by changes in the nonspecific turbidity of the reaction mixture, a problem occasionally encountered when the ordinary method is used (pretreated at 80°C). With the 70°C-pretreatment method, the nonspecific turbidity was eradicated and the values of (1→3)- β -D-glucan were lower than those obtained by the ordinary method. Thus, the 70°C-pretreatment method was less sensitive than the ordinary method, but its specificity was higher. The overall agreement rates between the kinetic turbidimetric assay and chromogenic assay were near 90%. Some samples in the control group showed unexpectedly high (1→3)- β -D-glucan values with the kinetic chromogenic assay. The measurement with the end-point chromogenic assay was influenced by sulfa drugs. In the kinetic turbidimetric method, the 70°C-pretreatment method is more effective than the ordinary method.

Key words : (1→3)- β -D-glucan, turbidimetric assay, nonspecific turbidity, influence of temperature, clinical evaluation.

Introduction

Methods for the measurement of (1→3)- β -D-glucan, a cell wall component of fungi, were developed for the diagnosis of fungal infections in Japan¹⁻⁵⁾. There are three commercially available methods: the end-point chromogenic *Limulus* test (FUNGITEC® G test, FUNGITEC® G test TE, [Seikagaku Corp., Tokyo, Japan])³⁾, the kinetic chromogenic *Limulus* test (FUNGITEC® G test MK, [Seikagaku Corp.])⁴⁾, and the kinetic turbidimetric *Limulus* test (β -Glucan test Wako, [Wako Pure Chemical Industries, Ltd., Osaka, Japan])⁵⁾. These methods are recognized as useful⁴⁻⁹⁾, although they cannot differentiate between fungal infections such as aspergillosis, candidosis, and pneumocystosis.

The β -Glucan test Wako based on the kinetic turbidimetric method is occasionally influenced by changes in the nonspecific turbidity of the reaction mixture. Abnormally high values are observed in a certain type of plasma with the ordinary method by dilution and heating at 80 °C for pretreatment. In our many clinical experiments, the nonspecific turbidity change of the reaction mixture was eliminated when the pretreatment was performed at a lower temperature.

Therefore, in this study, we compared the usefulness of the chromogenic methods (FUNGITEC G test and FUNGITEC G test MK) and kinetic turbidimetric methods (β -Glucan test Wako) using the pretreatments at 70°C and 80°C on the same samples, and we also clinically evaluated these four methods.

Methods

Criteria for diagnosis of fungal infections

Aspergillosis, candidosis, pneumocystosis, and

Address correspondence to: Takeshi Mori, M.D.

Division of Haematology, Department of Internal Medicine, Juntendo University School of Medicine, 2-1-1, Hongo, Bunkyo-ku, Tokyo, 113-8421, Japan.

cryptococcosis were diagnosed by the following criteria.

Aspergillosis: Definitive cases were diagnosed by (1) autopsy; and (2) a fever with no response to broad-spectrum antibiotics for a week or more, nodular or sharp-edged shadows on chest X-rays and CT-scan, and isolation of *Aspergillus* sp. from pleural effusion. Probable cases were diagnosed by a fever with no response to broad-spectrum antibiotics for a week or more; nodular shadows, infiltration or sharp-edged shadows or “halo” sign on chest X-rays; and positive (1→3)-β-D-glucan detection test and/or the positive Pastorex® *Aspergillus* test (Sanofi Diagnostic, Pasteur, Paris, France) with positive culture of *Aspergillus* sp. from sputum.

Candidosis (Candidemia): Definitive cases were diagnosed by (1) autopsy; and (2) a fever with no response to broad-spectrum antibiotics for a week or more, repeated isolation of *Candida* sp. from blood culture, and/or recognition of candidal endophthalmitis. Probable cases were diagnosed by a fever with no response to broad-spectrum antibiotics for a week or more, isolation of *Candida* sp. from blood culture once, and repeatedly positive (1→3)-β-D-glucan detection test and/or positive Pastorex® *Candida* test (Sanofi Diagnostic, Pasteur, Paris, France).

Pneumocystosis: Pneumocystosis was diagnosed by (1) autopsy; and (2) clinical symptoms including a fever with no response to broad-spectrum antibiotics for a week or more, dyspnea, cyanosis, hypoxia with alveolar-capillary block sign; diffuse infiltration or cloudy-glass shadows on chest X-rays; and detection of *Pneumocystis carinii* in the smear of sputa, bronchopulmonary lavage, or the polymerase-chain reaction method for the detection of *P. carinii*¹⁰⁾ performed at SRL, Inc.

(Tokyo, Japan).

Cryptococcosis: Cryptococcal meningitis was diagnosed by a fever without response to broad-spectrum antibiotics, clinical meningeal symptoms including headache and isolation of *Cryptococcus neoformans* from cerebrospinal fluid. Pulmonary cryptococcosis was diagnosed by fever, abnormal nodular shadow or cavitary lesion on chest X-rays, and isolation of *C. neoformans* from sputum and/or bronchoalveolar lavage.

Plasma from patients with fungal infections was obtained while the patients were being treated with antifungal agents in their acute phase.

Diseased control: Plasma from diseased controls with no evidence of fungal infection was obtained for the purpose of surveillance.

(1→3)-β-D-glucan measurement

The FUNGITEC G test [G test] and FUNGITEC G test MK [MK test] were used as the *Limulus* reagents for the end-point chromogenic and kinetic chromogenic methods, respectively. The cutoff value of the chromogenic methods was 20 pg/mL. β-Glucan test Wako was used as the *Limulus* reagent for the kinetic turbidimetric method with dilution and heating pretreatments at 70 °C [70 °C-pretreatment system] and at 80°C [80°C-pretreatment system]. The turbidity change of the reaction mixture was monitored by LABOSOFT-MT (Wako), a data acquisition program for the Toxinometer ET-251 (Wako). The cutoff value of the turbidimetric kinetic method was 11 pg/mL. For accurate measurement, each sample was divided into two and simultaneously, and then the two (1→3)-β-D-glucan values obtained were averaged.

The clinical diagnostic parameters were analyzed by the Chi-square test.

Table 1 Underlying diseases of patients tested by plasma (1→3)-β-D-glucan measurement

	Number of patients (Number of samples)														Total
	Underlying disease														
	AML	ALL	CML	NHL	MM	CHF	HCV	CD	DM	Parkin-sonism	MG	RD	Others		
Aspergillosis	2 (6)			1 (3)				1 (1)	1 (2)					5 (12)	
Definitive cases	1 (3)			1 (3)				1 (1)							
Probable cases	1 (3)								1 (2)						
Candidosis		1 (3)						1 (3)		2 (4)	1 (2)			5 (12)	
Definitive cases		1 (3)								1 (2)					
Probable cases								1 (3)		1 (2)	1 (2)				
Pneumocystosis				2 (2)				2 (4)						4 (6)	
Cryptococcosis				1 (2)		1 (3)	1 (3)						1 (2)	4 (10)	
Diseased control	1 (1)		1 (1)		1 (1)			4 (4)				2 (2)	3 (3)	12 (12)	
Total	3 (7)	1 (3)	1 (1)	4 (7)	1 (1)	1 (3)	1 (3)	8 (12)	1 (2)	2 (4)	1 (2)	2 (2)	4 (5)	30 (52)	

AML:Acute myelocytic leukemia, ALL:Acute lymphocytic leukemia, CML:Chronic myelocytic leukemia, NHL:Non-Hodgkin's lymphoma, MM:Multiple myeloma, CHF:Congestive heart failure, HCV:Hepatitis (HCV), CD:Collagen diseases, DM:Diabetes mellitus, MG:Myasthenia gravis, RD:Respiratory diseases

Results

The plasma samples examined were obtained from 35 normal healthy controls (35 samples), 12 diseased controls without fungal infections (12

Table 2 Plasma (1→3)- β -D-glucan values of healthy and diseased controls

Sample No.	Plasma (1→3)- β -D-Glucan Value (pg/mL)			
	β -Glucan Test Wako		FUNGITEC	
	70°C	80°C	G test	MK test
Healthy control				
1	< 3.2	< 3.2	0.9	5.4
2	< 3.2	4.0	1.5	5.6
3	< 3.2	< 3.2	1.4	4.3
4	< 3.2	3.5	1.0	7.5 (51.8)
5	< 3.2	3.5	3.6	6.5
6	< 3.2	< 3.2	1.9	11.1
7	< 3.2	< 3.2	0.6	5.2
8	< 3.2	5.1	2.1	5.2
9	< 3.2	< 3.2	2.6	2.9
10	4.3	5.4	0.1	3.0
11	< 3.2	3.8	2.2	8.5
12	< 3.2	< 3.2	0.8	5.8
13	< 3.2	< 3.2	1.6	6.6 (106.0)
14	6.0	6.1	4.9	9.5
15	3.7	5.1	2.5	14.1
16	< 3.2	< 3.2	1.6	8.5
17	3.6	4.0	2.3	9.5
18	3.4	4.6	2.6	12.5
19	9.8	11.4	12.5	21.8
20	4.4	4.7	4.1	13.4
21	< 3.2	< 3.2	1.1	10.2
22	5.8	7.7	2.4	18.1
23	< 3.2	4.3	2.8	11.2 (66.2)
24	4.3	5.3	2.6	11.1
25	< 3.2	< 3.2	0.6	2.8
26	< 3.2	< 3.2	7.5	9.2
27	3.3	< 3.2	0.8	13.2
28	5.8	< 3.2	2.1	0.0 (174.6)
29	< 3.2	< 3.2	8.1	2.0
30	< 3.2	< 3.2	2.2	4.2
31	3.2	3.2	2.2	4.2
32	< 3.2	< 3.2	2.2	0.0
33	< 3.2	< 3.2	2.8	0.0
34	3.3	4.0	3.0	4.5
35	< 3.2	< 3.2	3.2	0.0
Diseased control				
1	5.8	3.5	0.4	22.8
2	5.4	7.9	2.2	17.2
3	3.7	4.6	5.7	9.6
4	< 3.2	3.6	3.9	10.9
5	< 3.2	< 3.2	3.9	22.7
6	4.2	7.4	3.1	26.4
7	< 3.2	11.3	4.2	10.9
8	< 3.2	3.3	2.8	18.3
9	< 3.2	< 3.2	6.7	18.3
10	< 3.2	< 3.2	0.8	7.6
11	< 3.2	< 3.2	9.1	2.0
12	< 3.2	< 3.2	0.2	4.3

samples), 5 cases of systemic aspergillosis (12 samples), 5 cases of candidosis (12 samples), 4 cases of pneumocystosis (6 samples), and 4 cases of cryptococcosis (10 samples) (Table 1). These

Table 3 Plasma (1→3)- β -D-glucan value of systemic fungal diseases

	Plasma (1→3)- β -D-Glucan Value (pg/mL)			
	β -Glucan Test Wako		FUNGITEC	
	70°C	80°C	G test	MK test
Aspergillosis				
Definitive case				
Case 1.				
No. 1	7.7	11.5	2.4	11.8
No. 2	6.7	8.2	5.5	0.0
No. 3	< 3.2	4.6	9.4	28.1
Case 2.				
No. 4	101.9	104.3	324.5	460.6
Probable case				
Case 3.				
No. 5	1719.5	1755.5	3006.6	11694.0
No. 6	698.0	698.0	1533.3	3674.1
No. 7	155.7	153.5	214.8	432.3
Case 4.				
No. 8	< 3.2	< 3.2	5.0	11.4
No. 9	22.6	28.3	55.5	36.4
No. 10	11.5	13.0	149.8	197.7
Case 5.				
No. 11	12.0	34.5	3.5	30.6
No. 12	7.8	15.2	2.9	24.7
Candidosis (Candidemia)				
Definitive case				
Case 1. (<i>C. krusei</i>)				
No. 1	818.5	800.0	1344.4	3388.8
No. 2	99.6	112.0	74.4	166.7
No. 3	97.4	109.0	56.7	63.3
No. 4	482.1	506.5	522.4	1439.9
Case 2. (<i>Candida</i> sp.)				
No. 5	112.0	114.8	67.5	246.2
Probable case				
Case 3. (<i>C. albicans</i>)				
No. 6	56.5	76.4	61.2	109.9
No. 7	74.1	97.4	45.3	42.8
No. 8	35.2	48.8	25.7	28.4
Case 4. (<i>Candida</i> sp.)				
No. 9	222.9	247.5	191.5	151.1
No. 10	400.0	506.5	338.2	844.5
Case 5. (<i>C. glabrata</i>)				
No. 11	93.1	114.8	117.3	217.8
No. 12	44.6	56.5	30.8	116.7
Pneumocystosis				
Case 1.				
No. 1	247.5	287.1	488.6	408.5
Case 2.				
No. 2	8.7	11.5	81.2	71.7
Case 3.				
No. 3	24.8	27.7	21.7	73.6
No. 4	21.9	24.0	42.3	67.5
Case 4.				
No. 5	17.5	19.2	34.8	90.1
No. 6	4.3	5.4	20.6	2.5
Cryptococcosis				
Meningeal				
Case 1.				
No. 1	< 3.2	< 3.2	10.3	14.2
No. 2	< 3.2	< 3.2	1.9	26.3
No. 3	< 3.2	< 3.2	2.9	13.0
Case 2.				
No. 4	< 3.2	< 3.2	1.0	12.7
No. 5	7.4	3.5	1.6	12.8
Meningeal & pulmonary				
Case 3.				
No. 6	17.6	21.5	15.3	48.9
No. 7	17.8	20.3	11.1	37.0
No. 8	7.9	9.9	3.9	19.2
Pulmonary				
Case 4.				
No. 9	< 3.2	< 3.2	1.1	12.5
No. 10	< 3.2	< 3.2	22.7	24.7

() : isolated *Candida* sp. from blood culture

samples were stored at -80°C and liquefied at room temperature just before use. Four methods of $(1\rightarrow3)\text{-}\beta\text{-D-glucan}$ measurement were performed at same time.

The $(1\rightarrow3)\text{-}\beta\text{-D-glucan}$ values of the healthy controls and diseased controls are shown in Table 2. In 4 samples (Nos. 4, 13, 23, and 28), the plasma $(1\rightarrow3)\text{-}\beta\text{-D-glucan}$ values measured by the MK test were abnormally high in one of the data simultaneously measured and negative in the other. In this study, the lower value was used for calculation in all the healthy controls. As a result, one sample (No. 19) showed a positive value in both the MK test and 80°C -pretreatment system.

In the diseased controls, 1 sample (No. 42) examined by the 80°C -pretreatment system and 3 samples (No. 36, 40, and 41) measured by the MK test were positive, and all the other samples examined by the four methods were negative.

The plasma $(1\rightarrow3)\text{-}\beta\text{-D-glucan}$ values of patients with aspergillosis, candidosis, pneumocystosis, and cryptococcosis are shown in Table 3.

The nonspecific turbidity change was observed with the 80°C -pretreatment system in 3 samples (Nos. 4, 10, and 11) of aspergillosis, 1 sample (No. 5) of candidosis, and 1 sample (No. 1) of pneumocystosis, but it was not recognized by the 70°C -pretreatment system in any case. In the 5 samples in which the 80°C -pretreatment system showed the nonspecific turbidity change, the 70°C -pretreatment system detected lower values of plasma $(1\rightarrow3)\text{-}\beta\text{-D-glucan}$. These plasma samples were obtained from patients with acute myelocytic leukemia, diabetes mellitus, myasthenia gravis, and non-Hodgkin's lymphoma.

Tests on the 12 aspergillosis samples yielded positive results in 7 samples examined by the 70°C -pretreatment system, 9 samples examined by the 80°C -pretreatment system, 6 samples

examined by the G test, and 9 samples examined by the MK test.

In tests on the 12 candidosis samples, all samples examined by the four methods yielded positive results.

In pneumocystosis, positive results were obtained for all 6 samples by the G test, 5 samples by the MK test, 5 samples by the 80°C -pretreatment system, and 4 samples by the 70°C -pretreatment system. Among the 6 samples, 3 (Nos. 2, 4, and 6) were obtained during cotrimoxazole therapy.

In cryptococcosis (12 samples), positive results were obtained for 2 samples by the 70°C -pretreatment system, 2 by the 80°C -pretreatment system, 1 by the G test, and 4 by the MK test. The values of $(1\rightarrow3)\text{-}\beta\text{-D-glucan}$ examined by the 70°C -pretreatment system were noticeably lower than those examined by the 80°C -pretreatment system ($p=0.002$).

The positive rates of healthy controls, diseased controls, candidosis, aspergillosis, pneumocystosis, and cryptococcosis are shown in Table 4. In the diseased control group, the MK test showed a higher tendency than the 70°C -pretreatment system without significance in positive rate ($p=0.064$).

Table 4 Positive rates of plasma $(1\rightarrow3)\text{-}\beta\text{-D-glucan}$ measurement methods for fungal infection

	$\beta\text{-Glucan}$ Test Wako		FUNGITEC	
	70°C	80°C	G test	MK test
Normal control	0/35	1/35	0/35	1/35
Diseased control	0/12	1/12	0/12	3/12
Aspergillosis	7/12	9/12	6/12	9/12
Candidosis	12/12	12/12	12/12	12/12
Pneumocystosis	4/6	5/6	6/6	5/6
Cryptococcosis	2/10	2/10	1/10	4/10

Table 5 Comparison of diagnostic parameters of $(1\rightarrow3)\text{-}\beta\text{-D-glucan}$ measurement methods for fungal infections against the healthy controls

Disease	Method		True positive rate	True negative rate	Efficiency	Positive predictive value	Negative predictive value
Aspergillosis	$\beta\text{-Glucan}$	70°C	58.3	100	89.4	100	87.5
	Test Wako	80°C	75.0	97.1	91.5	90.0	91.9
	FUNGITEC	G test	50.0	100	87.2	100	85.4
		MK test	75.0	97.1	91.5	90.0	91.9
Candidosis	$\beta\text{-Glucan}$	70°C	100	100	100	100	100
	Test Wako	80°C	100	97.1	97.9	92.3	100
	FUNGITEC	G test	100	100	100	100	100
		MK test	100	97.1	97.9	92.3	100
Pneumocystosis	$\beta\text{-Glucan}$	70°C	66.7	100	95.1	100	94.6
	Test Wako	80°C	83.3	97.1	95.1	83.3	97.1
	FUNGITEC	G test	100	100	100	100	100
		MK test	83.3	97.1	95.1	83.3	97.1

Table 6 Comparison of diagnostic parameters of (1→3)-β-D-glucan measurement methods for fungal infections against healthy and diseased controls

Disease	Method	True positive rate	True negative rate	Efficiency	Positive predictive value	Negative predictive value
Aspergillosis	β-Glucan 70°C	58.3	100	91.5	100	90.4
	Test Wako 80°C	75.0	95.7	91.5	81.8	93.8
	FUNGITEC G test	50.0	100	89.8	100	88.7
	MK test	75.0	91.5	88.1	69.2	93.5
Candidosis	β-Glucan 70°C	100	100	100	100	100
	Test Wako 80°C	100	95.7	96.6	85.7	100
	FUNGITEC G test	100	100	100	100	100
	MK test	100	91.5	93.2	75.0	100
Pneumocystosis	β-Glucan 70°C	66.7	100	96.2	100	95.9
	Test Wako 80°C	83.3	95.7	94.3	71.4	97.8
	FUNGITEC G test	100	100	100	100	100
	MK test	83.3	91.5	90.6	55.6	97.7

Table 7 Comparison of overall agreement rates between different methods for (1→3)-β-D-glucan measurement

		70°C System		80°C System		G test		Overall agreement rates
		Positive	Negative	Positive	Negative	Positive	Negative	
G test	Positive	22	2					96.1%
	Negative	1	52					(70°C System vs. G test)
MK test	Positive	23	7					90.9%
	Negative	0	47					(70°C System vs. MK test)
80°C System	Positive	23	3					96.1%
	Negative	0	51					(70°C System vs. 80°C System)
G test	Positive			23	1			94.8%
	Negative			3	50			(80°C System vs. G test)
MK test	Positive			25	5			92.2%
	Negative			1	46			(80°C System vs. MK test)
MK test	Positive					23	7	89.6%
	Negative					1	46	(G test vs. MK test)

Tables 5 and 6 show the clinical diagnostic parameters of the four different methods of (1→3)-β-D-glucan measurement against the healthy controls, and against the healthy and diseased control groups combined, respectively.

Using healthy controls and diseased controls, the 70°C-pretreatment system and G test were significantly more reliable than the MK test in assessing the true negative rate for candidosis (p=0.041, p=0.041), as well as in overall efficiency in detecting candidosis (p=0.042, p=0.042). The 70°C-pretreating system and G test were significantly more reliable than the MK test in assessing the true positive rate (p=0.041, p=0.041) for aspergillosis.

Table 7 shows the overall agreement rates between the four (1→3)-β-D-glucan measurement methods in samples excluding cryptococcosis. The 70°C-pretreatment system had the highest rate of agreement with the 80°C-pretreatment system and G test in 96.1% of the

samples, while the agreement rate between the G test and MK test was low (89.6% of the samples).

Discussion

Fungal infections are strictly diagnosed when the fungus is isolated from a lesion without contamination, when a fungal lesion is pathologically recognized in the biopsy, or when the fungus is repeatedly detected in the blood or cerebrospinal fluid. In many cases, however, pathogenic organisms are not isolated, and this makes the diagnosis of opportunistic fungal infections difficult.

There are several available antigen detection methods in sera, including the polymerase-chain reaction test, (1→3)-β-D-glucan measurement methods, and so forth. For diagnosis of cryptococcosis, the Serodirect® "Eiken" *Cryptococcus* test (Eiken Chemical Co., Tokyo, Japan) and the Pastorex® *Cryptococcus* test (Sanofi Diagnostic, Pasteur, Paris, France) are commercially

available and have been shown to be useful.

For serological diagnosis of systemic candidosis, the CAND-TEC[®] test (Ramco Laboratories Inc. USA), the Pastorex *Candida* test, and method for the measurement of D-arabinitol¹¹⁾ in serum (Labofit, [Nacalai Tesque, Ltd., Kyoto, Japan]) are available. However, the CAND-TEC test is believed to have a high sensitivity but low specificity, and the Pastorex *Candida* test is known to have a high specificity but low sensitivity¹²⁾.

Just as in the case of the Pastorex *Candida* test in the diagnosis of candidosis, the Pastorex *Aspergillus* test has low sensitivity but high specificity in the diagnosis of systemic aspergillosis¹²⁾.

In pneumocystosis, the detection of *P. carinii* in the stained smear of sputum or by the polymerase-chain reaction method has proved to be useful.

While the method for the measurement of (1→3)- β -D-glucan in plasma cannot differentiate aspergillosis, candidosis, pneumocystosis, and so forth, it has been shown to be useful for diagnosis of fungal infections when clinical symptoms are considered. The two methods for (1→3)- β -D-glucan measurement, the chromogenic assay and turbidimetric assay, have their own merits and demerits. The substitutes for (1→3)- β -D-glucan used in these tests, i.e., pachyman for the chromogenic assay and curdlan for the turbidimetric assay, are fundamentally different. The differences between the chromogenic assay and turbidimetric assay are shown in Table 8.

A pretreatment of plasma is necessary to inactivate inhibitors in blood for the *Limulus* reaction. Current practical pretreatment methods are the dilution and heating method for the kinetic turbidimetric assay⁵⁾, the perchloric acid method for the end-point chromogenic assay³⁾, and the

alkaline method for the kinetic chromogenic assay⁴⁾. It is generally recognized that methods for the measurement of (1→3)- β -D-glucan show false-positive results in immunoglobulin products prepared with cellulose-based filter membranes¹³⁾. Furthermore, in our experiment, the change in the nonspecific turbidity was occasionally observed in samples from monoclonal gammopathy patients or myeloma patients, and the dilution and heating method yielded false-positive results. The change in nonspecific turbidity was probably caused by monoclonal γ -globulin in the plasma, a substance which could have changed in its conformation to construct an aggregated polymer during the pretreatment. We are going to evaluate our results on the influence of hyper- γ -globulin and may publish them in the near future.

The purpose of this study was to evaluate the new (1→3)- β -D-glucan measurement method with the 70°C-pretreatment system. Change in the nonspecific turbidity of the reaction mixture observed with the 80°C-pretreatment system disappeared when the 70°C-pretreatment system was used. The (1→3)- β -D-glucan values obtained with the 70°C-pretreatment system tended to be lower than those obtained with the 80°C-pretreatment system.

The overall agreement rates were near 90%, although the agreement between the values with the chromogenic assay and those with the kinetic turbidimetric assay were lower than expected.

In the control group, the MK test yielded high values of (1→3)- β -D-glucan in some samples. This may have been caused by a sampling error due to the small sample volume of only 5 μ l for this measurement. The influence of the change in nonspecific turbidity on the measurement with

Table 8 Differences of (1→3)- β -D-Glucan Measurement Methods

	FUNGITEC		β -Glucan Test Wako
	G test	G test MK	
Principle	End-point chromogenic assay	Kinetic chromogenic assay	Kinetic turbidimetric assay
β -Glucan standard	Pachyman		Curdlan + CM curdlan
Procedure	Microplate		Tube
Sample	Plasma or serum		Plasma or serum
Pretreatment	PCA method	Alkaline method	Dilution and heating method
Sample volume	100 μ L	5 μ L	100 μ L
Reaction time	30 min		90 min
Cutoff value	20 pg/mL		11 pg/mL

the MK test has not been discussed, and this point requires further study.

C. neoformans contain small amounts of (1→3)- β -D-glucan in their cell walls¹⁴⁾. One patient with cryptococcosis (Case 3) had concomitant infection with aspiration pneumonia including pulmonary candidosis. Because of this complication, positive rates of (1→3)- β -D-glucan in cryptococcosis were high. Therefore, in calculation of the diagnostic parameters the values obtained from cryptococcosis were excluded.

In contrast to the results obtained for the healthy controls, the diagnostic parameters of fungal infections showed, first, that 70 °C-pretreatment system is superior to the 80 °C-pretreatment system in patients with candidosis, and, second, that this system had slightly lower sensitivity but high specificity in patients with aspergillosis.

In contrast to the results obtained for the healthy and diseased controls, the diagnostic parameters of the four methods for the measurement of (1→3)- β -D-glucan showed similar tendencies (Table 4).

Sulfa drugs interfered with the (1→3)- β -D-glucan measurement by the G test using diazocoupling methods. Three samples of pneumocystosis (Nos. 2, 4, and 6) collected during co-trimoxazole therapy were positive by the G test but negative by the other three methods. According to these data, samples collected during co-trimoxazole therapy should not be examined by the G test.

In candidosis, all four methods showed good sensitivity and good specificity because *Candida* sp. were isolated from blood cultures of all cases and diagnosis of candidosis was consistently correct.

On the contrary, diagnosis of aspergillosis was difficult and the values of (1→3)- β -D-glucan were low in the early clinical course or when the patient was responding favorably to the antifungal therapy. Judging from these data, the sensitivity of these methods was thought to be low.

The cutoff value for the kinetic turbidimetric assay with the 80°C-pretreatment system was set at 11 pg/ml according to the previous study⁴⁾. In this study, we obtained comparatively lower (1→3)- β -D-glucan values with the 70 °C-pretreatment system than we did with the 80°C-pretreatment system, and the influence of nonspecific turbidity could be excluded by the 70°C-pretreatment system. Since the cutoff value was set for the two pretreatment temperatures, the 70°C-pretreatment system showed a slightly decreased sensitivity but its specificity rose to above

that of the 80°C-pretreatment system. Considering that the two methods have almost the same efficiency, the cutoff value of 11 pg/ml can be reliably used for the 70°C-pretreatment system.

In conclusion, the 70 °C-pretreatment system is more effective for the diagnosis of fungal infections because of its high specificity and elimination of the influence of changes in the nonspecific turbidity of the reaction mixture.

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