Studies on Immunodiagnosis of Visceral Mycoses Due to Opportunistic Pathogens

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Abstract

We are reporting the result of an investigation in immunodiagnosis of opportunistic fungus infections.

The number of patients who were detected with candidal mannan antigens and antibodies to candida were 8 (75%) and 3 (12.5%) in 24 patients with disseminated candidiasis or infection of the lung. Results of tests by two latex agglutination kits with sera obtained from patients with and without candidiasis recognized that the efficiency was 81.5% in kit A (mannan detection kit from Kyokuto Pharmaceutical Industrial Corporation) and 64.6% in kit B (intracytoplasmic protein detection kit from Ramco Laboratories), respectively.

In 11 cases with aspergilloma, aspergillus antibodies were detected in 10 cases (90.9%) by CIE and/or ID. All of 6 cases with invasive pulmonary aspergillosis were detected galactomannan antigen by using PASTOREX®-Aspergillus, while in 14 cases with clinically suspected aspergillus pneumonia, 3 cases (21.4%) were detected galactomannan antigen. Results of antigen detection tests in sera obtained from experimentally pulmonary aspergillosis in mice indicated that detection of antigen is very useful for rapid diagnosis of invasive pulmonary aspergillosis.

In 11 cases with cryptococcosis, all cases investigated were detected mannan antigen in sera and/or CSF by LA test kit from Eiken Co., Ltd.

Key words: Immunodiagnosis, Visceral Mycoses, Candidiasis, Aspergillosis, Cryptococcosis, Latex agglutination

Opportunistic fungus infections have been recognized as important infectious complication in compromised patients treated with immunosuppressive drugs/anticancer drugs/antibiotics. Especially, systemic infections caused by Candida species and invasive pulmonary aspergillosis and/or cryptococcosis have become a major cause of morbidity and mortality among hospitalized patients¹-⁴).

However, it is difficult to establish an antemorten diagnosis of these infections for several reasons. Namely, The clinical presentation is usually nonspecific and a microbiological diagnosis is often difficult.

From these present states, several techniques for detection on antigen and antibody and/or
fungal products\textsuperscript{5,6}) have been used: Counter-
immunoelectrophoresis (CIE)\textsuperscript{7}), Enzyme-linked
immunosorbent assay (ELISA)\textsuperscript{8,9}), Latex
agglutination\textsuperscript{10-12}), biochemical assay and
immunoblotting method\textsuperscript{14}) to diagnosis of
opportunistic fungal infections in recent years.

In this report, we describe the results of
investigation in immunodiagnosis of opportu-
nistic fungus infections, Candidiasis Aspergil-
losis and Cryptococcosis.

**Materials and Methods**

**Patients and Controls:**

Total 239 sera and 32 cerebrospinal fluid
(CFS) samples obtained from 54 cases with
candidiasis, 42 cases with pulmonary aspergil-
losis including clinical suspected cases, 11 cases
with cryptococcosis, 102 patients without
mycoses and 30 healthy persons were examined
in this study.

Of 17 cases with disseminated candidiasis
and 7 cases with pneumonia due to candida,
22 cases were diagnosed by histological exa-
minations in autopsies and 2 cases with can-
didemia were diagnosed by blood cultures and
clinical findings included the response against
antifungal therapy. In 30 cases with undis-
seminated candidiasis, a case and five cases
were oral candidiasis and candidal esophagitis
diagnosed by visual and endoscopical findings,
and 3 cases with candiduria were diagnosis
by cultural examinations (10^5/ml<). All of
the other case with undisseminated candidiasis
were autopsy cases.

In fourty two cases with pulmonary aspergi-
lliosis, 11 cases were allergic bronchopulmonary
aspergillosis (ABPA), another 11 cases with
aspergillumia, 6 cases with invasive pulmonary
aspergillosis confirmed diagnosted by demonstra-
tion of septate branched hyphae in pulmonary
tissue specimens or repeated isolation of
*Aspergillus fumigatus* from sputum with a
temperature greater than 38°C for more than 5
days which were unresponsive to antibacterial
agents with no evident etiology.

In 11 cases with cryptococcosis, 5 cases were
meningitis and 3 cases were cryptococcoma,
and 3 cases were meningitis with pneumonia
due to *Cryptococcus neoformans*. All of cases
with cryptococcosis were performed diagnosed
by cultural examinations.

**Preparation of self-made mannan antigens:**
*Candida albicans* serotype A human isolate
was used. Yeasts were fractured with glass
beads. The resultant material was centrifuged
at 10,000 \times g for 30 min. The pellected cell
wall fragments were then dried in acetone
and used for the preparation of mannan. The
supernatant crude cytoplasmic material was
ultracentrifuged at 100,000 \times g for 120 min to
remove organelles. This supernatant was then
tensively dialyzed in distilled water and
lyophilized. Mannan was removed by affinity
chromatography with concanavalin A-Sepharose
4B.

**Experimental pulmonary aspergillosis:**
Animal models were reported by Kume\textsuperscript{15})
for pulmonary aspergillosis in mice by means
of newly devised aerosol exposure apparatus.
Namely, after placing 12 mice pretreated with
cyclophosphamide and predonisolone in the
cages, setting them in the exposure apparatus
and sealing it hermetically, we put 18ml of
3.0 \times 10^8 cells/ml of *Aspergillus fumigatus*
(Kuboyama strain) suspended in 0.1% tween
80/physiological saline into a glass nebulizer
for feeding with a pressure of 1 Kg/cm^2 for
about 30 minutes. Once again we immediately
put 18ml of the conidia suspension into the
glass nebulizer, repeating the exposure. After
performing the exposure totally for 5 hours,
we removed the mouse cages and cleaned the
heads of the fixed mice with alcohol-moisture
cotton (mice without treatment). The treated
mice with antifungal drug were prepared with
inhalation of 500mcg/ml amphotericin B solu-
tion for 30 minutes after exposure of conidia
suspension totally for 5 hours by using the
same exposure apparatus.
Results

The comparison of detection between mannan antigen and antibody in 54 cases with candidiasis are shown in Table 1. The detection of candida antigen was performed by using Latex agglutination reagents from Kyokuto Pharmaceutical Industrial Corporation (LA-Kyokuto) and anticanalidial antibody was detected by counterimmunoelectrophoresis (CIE) and Immunodiffusion test (ID) by using self-made antigen. The number of patients detected with candidal mannan antigens and anticanidial antibodies were 18 (75.0%) and 3 (12.5%) in 24 cases with disseminated candidiasis or infection of the lung, respectively. While, the number of antigen and antibody positive cases were 4 (13.3%) and 13 (43.3%) in 30 cases with infection of the digestive tract and/or the urinary tract, respectively.

The positive cases of candidal mannan antigen detected by LA-Kyokuto are summarized in Table 2. by each clinical group.

The No. of positive case in cases with disseminated candidiasis or infection of the lung was 18 cases (75.0%). While, in case with infection of the digestive tract or urinary tract and/or oral cavity, 4 cases (13.3%) were detected with candidal mannan antigens by LA-Kyokuto. In 30 cases with other visceral fungal infections (28 cases of pulmonary aspergillosis including the suspected cases, 10 cases of cryptococcosis, a case of mucormycosis and a case with systemic infection due to Trichosporon beigelii) and cases without mycoses and healthy control, candidal mannan antigens were not detected.

The efficiencies were compared between Kit A, namely, mannan antigen detection kit (LA-Kyokuto) and Kit B, namely, intracytoplasmic protein antigen detection kit (Cand-Tec) from Ramco Laboratories. The results of tests by these two candidal antigen detection kits with sera from patients with and without candidiasis are shown in Table 3.

The sensitivity and the specificity of kit A were 60% and 100%, and those of kit B were 60% and 68.6%. One hundred percent of patients with visceral candidiasis and 64.5% of patients without candidiasis were diagnosed by kit A correctly. However, in the case of kit B, 62.1% of the cases with candidiasis and 66.7% of cases without candidiasis were predicted. From these results, the efficiency was 81.5% in kit A and 64.6% in kit B.

The comparison between detection of garactomannan antigen and antibody in patients with ABPA, Aspergilloma, invasive pulmonary aspergillosis or clinically suspected aspergillus pneumonia are summarized in Table 4.

Latex tests for detection of aspergillus galactomannan were performed by using PASTOREX® Aspergillus (sensitivity : 15ng/ml of galactomannan) and CIE and/or ID system were used for detection of aspergillus antibodies by the using self-made antigen obtained from Asper-

Table 1. Comparison of detection for mannan antigen and antibody to patients with Candidiasis

<table>
<thead>
<tr>
<th>Patients</th>
<th>Antigen*</th>
<th>Antibody**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disseminated Candidiasis and infection of the lung (n=24)</td>
<td>18 (75.0)</td>
<td>3 (12.5)</td>
</tr>
<tr>
<td>Infection of the digestive tract and/or the urinary tract (n=30)</td>
<td>4 (13.3)</td>
<td>13 (43.3)</td>
</tr>
</tbody>
</table>

Note; Data express the number of positive cases (%)
*Latex tests for antigenemia were performed by using the LA test reagents from Kyokuto Pharmaceutical Industrial Co., Ltd.
**Counterimmunoelectrophoresis and immunodiffusion were used for detection of candidal antibody by self-made antigen obtained from Candida albicans.
gillus fumigatus.

Galactomannan antigen was not detected in the serum obtained from the case with ABPA or aspergilloma. In 11 cases with ABPA, 4 cases (36.4%) were detected aspergillus antibodies by means of CIE, and aspergillus antibodies were detected in 10 cases (90.9%) of the case with aspergilloma by the CIE and the ID. While, all cases with invasive pulmonary aspergillosis were detected galactomannan antigen without detection of antiaspergillus antibodies. In 14 cases with clinically suspected aspergillus pneumonia, 3 cases (21.4%) and 2 cases (14.3%) were detected galactomannan antigen and antibody, respectively.

Fig. 1 demonstrate the changes in the number of viable fungal cells in the lung, the amount of intrapulmonary fungus cells, and the time course changes in the severity of pulmonary lesions which were evaluated from the area of the infected lesions and the number of lesions in the comparison between treated mice and untreated mice.

In the mice treated with AMPH, the number of viable fungal cells in the lung was $3.9 \times 10^4$/left lung, immediately after the exposure, $3.2 \times 10^4$/left lung after 2 hours, $5.9 \times 10^3$/left lung after 8 hours, 0 after 24 hours and 48 hours.

Table 2. Positive rate of Candida mannan antigen detected by Kyokuto LA kit in each clinical groups

<table>
<thead>
<tr>
<th>Clinical group and focus of infection</th>
<th>No. of cases</th>
<th>No. of positive cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I. Candidiasis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;Group A&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systemic candidiasis</td>
<td>4</td>
<td>3 (75.0%)</td>
</tr>
<tr>
<td>Candidemia</td>
<td>9</td>
<td>8 (88.9%)</td>
</tr>
<tr>
<td>Lungs</td>
<td>7</td>
<td>5 (71.4%)</td>
</tr>
<tr>
<td>Lungs and digestive tract</td>
<td>2</td>
<td>1 (50.0%)</td>
</tr>
<tr>
<td>Lungs and urinary tract</td>
<td>2</td>
<td>1 (50.0%)</td>
</tr>
<tr>
<td>sum</td>
<td>24</td>
<td>18 (75.0%)</td>
</tr>
<tr>
<td>&lt;Group B&gt;</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Digestive tract (severe)</td>
<td>3</td>
<td>2 (66.7%)</td>
</tr>
<tr>
<td>Digestive tract (severe) &amp; urinary tract</td>
<td>2</td>
<td>1 (50.0%)</td>
</tr>
<tr>
<td>Digestive tract (mild)</td>
<td>15</td>
<td>0</td>
</tr>
<tr>
<td>Digestive tract (mild) &amp; urinary tract</td>
<td>3</td>
<td>1 (33.3%)</td>
</tr>
<tr>
<td>Urinary tract</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Urinary tract and oral cavity</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>Oral cavity</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>sum</td>
<td>30</td>
<td>4 (13.3%)</td>
</tr>
<tr>
<td>II. Other visceral fungal infections*</td>
<td>40</td>
<td>0</td>
</tr>
<tr>
<td>III. Patients without mycoses (including Candida colonization)</td>
<td>102</td>
<td>0</td>
</tr>
<tr>
<td>IV. Healthy controls</td>
<td>30</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>226</td>
<td>0</td>
</tr>
</tbody>
</table>

Note: *28 cases with pulmonary Aspergillosis, 10 cases with pulmonary Cryptococcosis, 1 case of Mucormycosis and 1 case of Trichosporon beigelii infection.
and 6.9×10^2/left lung after 72 hours, whereas the mice untreated with AMPH shows the larger number of viable fungal cells in the lung, such as 7.9×10^4/leaf lung immediately after exposure, 4.2×10^4/leaf lung after 2 hours, 2.1×10^4/leaf lung after 8 hours, 8.0×10^3/leaf lung after 24 hours, 2.2×10^3/leaf lung after 48 hours and 3.2×10^2/leaf lung after 72 hours. While the treated mice showed a tendency for the viable fungal cells to disappear from the lung in 48 hours after the exposure and formed mild infected lesions in 72 hours after the exposure, the untreated mice demonstrated a tendency for the number of viable fungal cells in the lung to conspicuously increase in 72 hours after the exposure and formed infected lesions in 24 hours after the exposure. At 72 hours to 96 hours after the exposure, the latter

<table>
<thead>
<tr>
<th>With Candidiasis</th>
<th>Kit A</th>
<th>18 (a)</th>
<th>12 (b)</th>
<th>30 (c)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Kit B</td>
<td>18 (a')</td>
<td>12 (b')</td>
<td>30 (c')</td>
</tr>
<tr>
<td>Without Candidiasis</td>
<td>Kit A</td>
<td>0 (d)</td>
<td>35 (e)</td>
<td>35 (f)</td>
</tr>
<tr>
<td></td>
<td>Kit B</td>
<td>11 (d')</td>
<td>24 (e')</td>
<td>35 (f')</td>
</tr>
<tr>
<td>Total</td>
<td>Kit A</td>
<td>18 (g)</td>
<td>47 (h)</td>
<td>65 (i)</td>
</tr>
<tr>
<td></td>
<td>Kit B</td>
<td>29 (g')</td>
<td>36 (h')</td>
<td>65 (i')</td>
</tr>
</tbody>
</table>

Note: *Kyokuto LA Kit, **Cand-Tec LA Kit
- Sensitivity; Kit A (a/c) = 60%, Kit B (a'/c') = 60%
- Specificity; Kit A (e/f) = 100%, Kit B (e'/f') = 68.6%
- Predictive value positive; Kit A (a/g) = 100%, Kit B (a'/g') = 62.1%
- Predictive value negative; Kit A (e/h) = 74.5%, Kit B (e'/h') = 66.7%
- Efficiency; Kit A (a+e/c+f) = 81.5%, Kit B (a'+e'/c'+f') = 64.6%

Table 4. Comparison of detection for mannan antigen and antibody to patients with ABPA, Aspergilloma and Invasive pulmonary Aspergillosis

<table>
<thead>
<tr>
<th>Patients</th>
<th>Antigen*</th>
<th>Antibody**</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABPA (n=11)</td>
<td>0 (0.0)</td>
<td>4 (36.4)</td>
</tr>
<tr>
<td>Aspergilloma (n=11)</td>
<td>0 (0.0)</td>
<td>10 (90.9)</td>
</tr>
<tr>
<td>Invasive pulmonary Aspergillosis (n=6)</td>
<td>6 (100.0)</td>
<td>0 (0.0)</td>
</tr>
<tr>
<td>Clinically suspected Aspergillus pneumonia (n=14)</td>
<td>3 (21.4)</td>
<td>2 (14.3)</td>
</tr>
</tbody>
</table>

Note: Data express the number of positive cases (%)
- ABPA: Allergic bronchopulmonary aspergillosis
- * Latex tests for aspergillus galactomannan were performed by using PASTOREX® Aspergillus. (sensitivity: 15 ng/ml of galactomannan)
- ** Counterimmunoelectrophoresis and fungal immunodiffusion system were used.
mice demonstrated a mortality rate of 80% to 100%.

Table 5 showed the antigen titer detected by using PASTOREX-Aspergillus for galactomannan in sera obtained from experimentally pulmonary Aspergillosis in mice. Galactomannan antigen in sera was detected at 48 hours after the exposure of Aspergillus fumigatus. These results indicate that detection of antigen is very useful for rapid diagnosis of invasive pulmonary aspergillosis.

The results of tests for the detection of Cryptococcal antigen and antibody in sera and CSF from patients with Cryptococcosis are shown in Table 6. In 11 cases with cryptococcosis, namely 5 cases with meningitis, and 3 cases with meningitis and pneumonia, and 3 cases with pneumonia, mannan antigen was detected in all cases investigated using the LA test kit (from Eiken Co., Ltd.) after protease
treatment, and the titers ranged from 1:8 to 1:2048. 3 (30%) of 10 patients investigated were antibody-positive by CIE which was performed by 200mcg/ml of crude polysaccharide prepared from Cryptococcus neoformans serotype A.

Discussion

Despite increasing clinical awareness of candida infections, clinical and conventional microbiologic diagnosis remains difficult. Therefore, a number of methods for rapid serologic diagnosis of significant candida infection have been investigated. Detection tests for antibody to candida antigens have been widely studied16), but the diagnostic efficiency of detection of candida antibody remains controversial17). Measurement of candida antigens or metabolites has therefore been attempted5,6,18,19).

Two latex agglutination kits to detect candida antigen were compared for the diagnostic efficiency in theses studies. We chose a latex agglutination test for detection of antigen because of the simplicity of this method and, therefore, its practicality for widespread application. The efficiency and specificity were 81.5% and 100% in mannan detection kit from Kyokuto Co., Ltd. (LA-Kyokuto) and 64.6% and 68.6% in intracytoplasmic protein detection kit from Ramco Lab. (Cand-Tec). These results indicate detection of mannan dissolved from the candida surface and circulating in serum by using LA-Kyokuto has been diagnostically useful. But since some serum samples from disseminated candidiasis were positive only by LA-Kyokuto and some other samples were positive only by Cand-Tec, a combination of another techniques, Cand-Tec, may prove more useful.

We investigated the evaluation of LA test to detect candidal mannan antigen in sera obtained from experimental gastric candidiasis of mice with or without treatment. The result shows good correlation between the change of the titer of mannan antigen and the severity of infections, and gastric lesions healed histopathologically 3 weeks after disappearance of mannan antigen in sera obtained from mice treated with antifungal agent. From these results we emphasize that the antifungal

<table>
<thead>
<tr>
<th>Case</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Sera Ag</th>
<th>Ab</th>
<th>CSF Ag</th>
<th>Ab</th>
<th>Latex agglutination for detection of antigen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>78</td>
<td>F</td>
<td>meningitis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>−</td>
<td>×2048 ×1024</td>
</tr>
<tr>
<td>2</td>
<td>62</td>
<td>F</td>
<td>pneumonia</td>
<td>+</td>
<td>−</td>
<td>+</td>
<td>−</td>
<td>×1024 ×4096</td>
</tr>
<tr>
<td>3</td>
<td>68</td>
<td>F</td>
<td>meningitis</td>
<td>NT</td>
<td>NT</td>
<td>−</td>
<td>−</td>
<td>NT ×16</td>
</tr>
<tr>
<td>4</td>
<td>39</td>
<td>F</td>
<td>meningitis</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>×32 ×32</td>
</tr>
<tr>
<td>5</td>
<td>27</td>
<td>F</td>
<td>pneumonia &amp; meningitis</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>−</td>
<td>×32 NT</td>
</tr>
<tr>
<td>6</td>
<td>33</td>
<td>M</td>
<td>pneumonia</td>
<td>+</td>
<td>−</td>
<td>NT</td>
<td>NT</td>
<td>×128 NT</td>
</tr>
<tr>
<td>7</td>
<td>60</td>
<td>M</td>
<td>pneumonia</td>
<td>−</td>
<td>−</td>
<td>NT</td>
<td>NT</td>
<td>×64 NT</td>
</tr>
<tr>
<td>8</td>
<td>53</td>
<td>M</td>
<td>meningitis</td>
<td>−</td>
<td>−</td>
<td>NT</td>
<td>NT</td>
<td>×32 ×8</td>
</tr>
<tr>
<td>9</td>
<td>84</td>
<td>M</td>
<td>pneumonia &amp; meningitis</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td>×512 ×1024</td>
</tr>
<tr>
<td>10</td>
<td>26</td>
<td>M</td>
<td>pneumonia &amp; meningitis</td>
<td>−</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT NT</td>
</tr>
<tr>
<td>11</td>
<td>21</td>
<td>F</td>
<td>meningitis</td>
<td>NT</td>
<td>NT</td>
<td>−</td>
<td>−</td>
<td>×32 ×256</td>
</tr>
</tbody>
</table>

Note; Latex tests for antigenemia were performed by using the serodirect "Eiken" (sensitivity: 6.25ng/ml)
therapy is necessary for about more 3 weeks since candidal mannan antigen disappeared from sera.

Pulmonary aspergillosis, especially invasive pulmonary aspergillosis are being recognized with increasing frequency \(^{20,21}\). Rogers et al\(^{22}\) reported ELISA for detection of aspergillus antigen gave positive and negative predictive values for invasive pulmonary aspergillosis of greater than 95%, demonstrating the value of antigen detection in early diagnosis of invasive pulmonary aspergillosis infection. However, ELISA or RIA procedures are not commercially available.

A recent assay system which is technically simpler has been marketed by Diagnostics Pasteur, namely, PASTOREX\(^{\circledR}\)-Aspergillus. This assay system is based on latex particles coated with rat monoclonal antibody prepared against galactomannan. Sensitivity of the reagent is 15ng/ml. Dupon et al\(^{23}\) reported sensitivity and specificity of the PASTOREX\(^{\circledR}\)-Aspergillus was 93.3% and 100% in prospective studies used sera obtained from the patient with or without invasive aspergillosis. While, Haynes et al\(^{24}\) indicated sensitivity of 94.7% and specificity of 93.2% in their retrospective studies.

In our own investigation with this new kit, galactomannan antigen was not detected in the serum obtained from the case with ABPA or aspergilloma. While, in all of the 6 cases with invasive pulmonary aspergillosis, galactomannan antigen was detected. In addition, galactomannan antigen in sera obtained from experimental pulmonary aspergillosis in mice by using this kit was detected in early stage of infection. These results indicate PASTOREX\(^{\circledR}\)-Aspergillus is very useful for rapid diagnosis of invasive pulmonary aspergillosis.

*Cryptococcus neoformans* is an encapsulated, yeast-like pathogen with world wide distribution. This fungus is recognized as the etiologic agent of cryptococcosis and most often is manifested as a pulmonary or meningeal infection \(^{25}\). The latex test for cryptococcal antigen detection is one of the most reliable fungal serological tests available to clinicians. Especially it is most useful for the diagnosis used CSF of cryptococcal meningitis, but circulating capsular polysaccharide antigen can sometimes be detected in the serum of patients having other forms of cryptococcosis. In our studies, mannan antigen in the serum by LA test kit (from Eiken Co., Ltd.)\(^{26}\) was detected in all cases with cryptococcosis investigated.

The detection of cryptococcal antigen in serum is, however, some times obscured by the presence of rheumatoid factor (RF) or other interfering substances\(^{27}\). When this occurs, it is difficult to determine whether the serum contains cryptococcal antigen, interference factors, or both. In 1983 Stockman and Robert\(^{28}\) used a protease treatment to destroy interfering factors in the LA test, and indicated that protease have enough ability to remove interfering factors. Moreover, protease pretreatment may have released antigen from soluble immune complexes in sera. The LA kit from Eiken dose actually contains the protease pre-treatment procedure, therefore high sensitivity and specificity and/or high antigen titer would be obtained in our investigations.

In 1935, Benham\(^{29}\) divided *Cryptococcus neoformans* into two serologic sub group. Evans\(^{30}\) later divided it into three serotypes, and Walter and Coffee\(^{31}\) reported a fourth serotype in 1968. These four serotypes, designated as A, B, C and D, are defined by specific rabbit antisera, preabsorbed with strains of the heterologous groups to remove cross-reactivity.

While, Shinoda et al\(^{32}\) have been described the serotype distributions of *Cryptococcus neoformans* isolated clinical specimens were 87% for A, 9% for D and 4% for AD, and serotype B and C have not been isolated in Japan. From these results, this LA kit was prepared with antibody immunized by *Cryptococcus neoformans* serotype A\(^{26}\). It is natural results (unpublish data) that the antigen titer by using above the LA kit has been recognized higher titer in sera obtained from mice infected with *Cryptococcus neoformans* serotype A than
that infected with serotype B, C or D.

References


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