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RAPID DIAGNOSTIC METHOD FOR FUNGI BY MEANS OF FLUORESCENT ANTIBODY TECHNIQUE

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INTRODUCTION

After the fluorescent antibody technique was first introduced into the field of chemical immunology in 1941 by A.H. Coons [1 - 3], gradually its application was tried in other areas and in this the evaluation of the method in actual use for the diagnosis of infection has been emphasized. Such matters as the distribution of antigens and antibodies in the structures [4-17], their analysis [18-20], as well as the rapid diagnosis of bacterial smear samples [21-29] has become widespread.

Today many classifications of fungi and revisions of existing analytical methods have occurred and results of serological diagnostic methods [30-33] have been reported which are superior to those results obtained by the procedures of Martin-Jones [34] and Loder Van Rij [35].

Using the fluorescent antibody technique as a serological procedure diagnosis of fungus was tried. Fundamental and clinical trials were conducted for rapid analysis of smear samples or for internal structure and although the results have been reported previously. I would like to report and discuss with you members the experimental methods and results in actual use.

The principle of the fluorescent antibody technique is that the specific immune serum obtained from an immuno-chemical reaction reacts specifically and selectively with an antigen due to a label previously placed on this serum and therefore at this point only a specific portion reacts. Moreover, under specific conditions the united part is scrutinizable and were thus examined. Under these circumstances it is of course

necessary to have a high titer for the specific immune-serum. Moreover the label must be given in such a manner that the characteristic is not destroyed.

I. THE PREPARATION OF VARIOUS TYPES OF SPECIFIC IMMUNE SERUM AND OF FLUORESCENT LABELLED ANTIBODIES FROM CANDIDA GENUS

In the preparation of specific immune serum, either heated antigen or simply heated antigen is satisfactory. Although the difference between the two is minimal and high titers for the serum can be obtained, nevertheless it is thought that the former is slightly superior with respect to ill effects to the immune animals.

1. Heated Antigen and Method of Immunization

The antigens used for immunization were the standard stock cultures maintained at the Piltz Center of Juntendo University and included: Candida albicans, ATCC. 10259, C. pseudotropicalis NAGAO 7494, C. guilliermondii. NAGAO. 7335, C. Scottii. IFO. 0736, C. catenulata. IFO. 0720, C. krusei. NAGAO. 7492, C. parakrusei. ATCC. 10264, C. stellatoidea ATCC. 7349, C. tropicalis, ATCC. 7349, Saccharomyces rosei, IFO. 0428, S. bisporus IFO. 0723, Torulopsis glablata. IFO. 0005. Each standard stock culture was grown at 37°C for 48 hours in Sabouraud's agar medium with yeast added. Then after collecting and washing, a suspension containing 20 - 30 mg/ml of yeast was made, heated at 100°C for 2.5 hours andused as the immunizing antigen.

The animals used for immunization were domestic rabbits of 2.5-3.0 kg body weight. Those Candida varieties which did not previously cause agglutination reaction will be the subject matter.

As illustrated in Table 1, in theimmunization procedure the heated antigen suspension was normally injected in a series of 3 or 4 shots. Then after 10 - 14 days subsequent to the final injection, blood was sampled from all and according to the calculated agglutination reaction, the titer was determined. That is to say, each immunizing antigen was used, diluted and mixed with the immune serum, maintained at 37°C in a constant temperature bath for two hours, then after storing overnight in a cold room the agglutination titer was read.

2. Preparation of Specific Immune Serum

The immune serum obtained from each species of Candida mutually possessed the common antigen (factor) and did not demonstrate specific characteristics whatsoever. Thus there was a necessity to eliminate the common factor. A 1:10 dilution of Candida antisera having an agglutinating titer above 1:1280 was made, and in the proportion of 1-5 mg of specifically chosen adsorbing heat treated yeast in 1 ml of diluted serum,

Table 1 Immunization Method

1)日 2)加热抗原		1)日	2)加热抗原
1	0.5 ml	7	2.0 ml
2	1.5	8	—
3	1.5	9	2.0
4	—	10	2.0
5	2.0	11	2.0
6	2.0		

3) 家兔体重: 2.5~3.0 kg

1) Days
2) Heated antigen
3) Weight of domestic rabbit 2.5 - 3.0 kg

the common factor was eliminated. The suspension containing adsorbing yeast was kept at 37°C - 52°C in a constant temperature bath for two hours and maintained overnight in a cold room. The supernatant was separated and according to the agglutination reaction on a slide the serum was examined for non-specific agglutination using the heat treated fungus from each species or live yeast as antigens. If they remained they were readsorbed; adsorption normally occurred 3 - 4 times and again the readsorption was repeated with other specified Candida species.

For example, the result of establishing the agglutination titer of the antiserum from four varieties of Candida obtained after immunization (Candida albicans, C. stellateidea, C. guilliermondii, Torulopsis glablata) was that the C. albicans group exhibited 640 - 1280 fold titer. C. guilliermondii group 640 - 1280 fold, C. stell group 1280 fold, T. glablata group 640 - 1280 fold, or values greater than given were obtained for each immune serum. These sera were thermally inactivated (56°C for 30 minutes) and 5% phenol at a proportion of 0.5% was added. The 1280 fold sera were diluted 10 fold and the slide agglutination reaction was performed for each species of Candida, Torulopsis, Saccaromyces and Cryptococcus. The C. albicans, C. stell., C. guill. and T. glablata antisera have, with the exception of Cr. neoformans, strong differences toward Candida, Torulopsis and Saccaromyces; but demonstrated an agglutination reaction. In contrast the spontaneous agglutination with physiological saline did not occur. The foregoing anti-Candida sera mutually possessed with all species the common factor and did not show any specificity. Now, as illustrated in Table 2, C. trop. as the adsorbing yeast, adsorption with C. albicans antiserum was performed three times; however, C. stell., C. pseudotropicalis, T. glablata

Table 2 Adsorption Method

1)免疫血清	2)吸収加熱菌
C. albicans	C. tropicalis C. pseudotropicalis
C. guill.	C. albicans C. krusei
C. stell.	S. pasterianus C. albicans C. pseudotropicalis
T. glab.	C. albicans S. bispor (C. krusei)

1) Immune serum
2) Adsorbing thermally treated fungi

and S. bisporus demonstrated agglutination to a slight degree. When C. pseudotropicalis was used and adsorption was performed, the specific immune serum with respect to C. albicans was obtained. However, despite thorough adsorption, there were antisera which could not be partitioned by adsorption.

With C. guill antiserum and with T. glablata antiserum, C. albicans and S. bisporus were adsorbed several times; then with each C. krusei was sufficiently adsorbed and thus the objective of four specific immune sera was attained. From these facts whether there were differences in the individual immunized rabbits, the difficulties applied to the elimination of non specific factors were investigated. Even with three and four repeated adsorptions, no adsorption occurred. There are many instances where adsorption was attained by using immune sera from other rabbits. The other antisera were comparatively easily adsorbed.

3. Preparation of Fluorescent Labeled Antibodies

In order to avoid diluting the fluorescent labelled antibody containing large amounts of inactive proteins in the immune serum, it is necessary to eliminate serum albumin and this was accomplished by the ammonium sulfate salting out procedure of Coons [37]. That is to say, a minimum of 30 ml of high titer antiserum was cooled, a similar quantity of

saturated ammonium sulfate added and this cloudy solution was centrifuged at 4°C. The sediment was again washed with one half saturated ammonium sulfate, and again centrifuged and washed. Subsequently the sediment was dissolved in buffered saline solution of 1/5 volume of the original serum solution and subjected to cellophane dialysis in a cold room until NH_4 [sic] was no longer detectable. Fluorescent dye was combined with this globulin solution or serum. That is to say, the above mentioned γ-globulin or serum was diluted with 0.15 M saline solution containing a 0.5 M pH 9.0 buffer of $NaHCO_3$ - Na_2CO_3 so that the protein concentra tion was 1 - 2%. Determination of protein concentration was made with the Micro-Kylder method. For example, in the preparation of 50 ml of fluorescent labelled antibody solution, in order to adjust 45 ml of a 1% antiserum or δ-globulin solution, 22.5 ml of 0.15 M saline solution as well as 5 ml of 0.5 M carbonate bicarbonate buffer at pH 9.0 are added to 22.5 ml of 2% γ-globulin. This combined solution was cooled to approximately 4°C and with respect to 1 mg % protein quantity a ratio of 0.05 mg dye was added and stirred for 12 - 18 hours at 4°C. At first the pigment floats on the solution surface but gradually a fluorescent labelled antibody solution is formed which strongly emits a yellow green fluorescence. After Riggs synthesized the fluorescent dyes, fluorescein isothiocyanate and Rhodamine B isothiocyanate in 1958, their convenience and stability were shown and have become widely used. These isothiocyanated dyes, fluorescein isothiocyanate and Rhodamine B isothiocyanate, manufactured by Baltimore Biological Laboratory Inc. as well as the Sheep Anti Rabbit Globulin Fluorescein conjugate manufactured by Sylvana were used in the experiments.

The excess fluorescent dye and nonspecific fluorescent material were eliminated according to the method of Marshall using cellophane dialysis. Although the fluorescent antibody solution reacts with a component of the normal structure and gives rise to the spontaneous formation of a greenish yellow color, the properties of this material are not yet known. However, with the adsorption treatment of acetone on the visceral powder, the spontaneous fluorescence can be prevented to a certain extent. That is to say in the preparation of acetone dried structural powder 25-30 mg of fresh mouse liver was homogenized with a similar amount of 0.15% normal salt solution, treated with acetone, passed through a Burthner funnel anddried; this powder in a proportion of 100 mg/ml is agitated for adsorption with the fluorescent antibody solution (usually 5 ml), sufficient adsorption is made, centrifuged in thecold and a preservative added after the clear filtrate was separated. From the foregoing process the objective, fluorescent labelled antibody was finally obtained.

II DIAGNOSIS OF SMEARED FUNGUS SPECIMEN

1. Method and Materials

In themanufacture smear specimens in order that the spontaneous fluorescence would occur, glass plate of good quality (Micro Stand rd

Naturamiglass) and as thin as possible (1 mm $\pm$ 0.2 mm) was used. The smear was made using a sterilized platinum loop, fungi were fished from typical colonies of each variety, smeared on a well-polished, grease-free glass plate on which was placed a drop of saline solution. Fixation was carried out variously with ethanol, formalin, acetone or flame treatment.

In the fluorescent antibody method, there are the direct, indirect. and complement techniques. The direct and indirect methods were primarily used. The equipment consisted of - a simple humidity cabinet, a large sized "sharley" with a cover (within which a moistened filter paper is left), indicator vial, pipette, buffer as well as saline glycerol buffer (mixture of 18 ml purified glycerol and 2 ml buffered saline), all species of labelled anti-Candida solutions or sera and a fluorescence microscope (OSRAM HBO-200).

In the direct method, after fixation of a sample, simple washing with buffer saline, several drops of fluorescent labelled anti-Candida serum or γ-globulin solution was added as illustrated in Table 3, placed in the aforementioned large sized "sharley", reacted sufficiently for 20-30 minutes at 37°C, then later excess antibody was washed with buffered saline, again placed in an indicator vial and thoroughly washed with buffered saline while being impacted for 15 minutes. The moisture outside the smear was wiped off and with one drop of buffered glycerol it was enclosed with a cover glass, then microscopically analyzed.

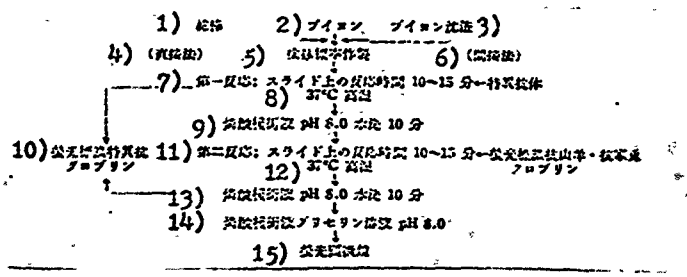
In the indirect method, first when the un-labelled antibody was treated as a layer, the antigen on the sample reacted with the component of the antibody and become adsorbed. Excess antibody was removed by washing and the combined γ- globulin was left. Since this γ-globulin still has the antigenic characteristics, the method utilized this as the antigen for detection. Using the specific anti-Candida solution obtained from domestic rabbits, labelled solutions from goat and anti rabbit γ-globulin, these were two-fold dyed following Table 3 then microscopically examined.

In order to utilize the fluorescent antibody method it is important to separate the specific and non-specific reactions; in the direct as well as the indirect methods, on each occasion the contrast was differentiated without fail and the distinction was made. That is to say, in the direct method the inhibition experiment was used wherein the prevention of the specific reaction caused by the same type of antibody which was not labelled and where prevention of specificity due to a different type of antibody did not arise. Even in the indirect method, by interchanging layers of various antibodies and normal sera, the separation of specificity or non-specificity could be detected.

2. Results

Identification of Candida albicans. With respect to fluorescent

Table 5 Fluorescent Antibody Reaction On Smeared Samples



1) Cotton swab
2) Broth
3) Broth sediments
4) (Direct method)
5) Preparation of smear sample
6) (Indirect method)
7) First reaction; Reaction time on slide 10-15 minutes ← Specific antibody
8) 37°C high temperature
9) Phosphoric acid buffer pH 8.0 Water wash 10 minutes
10) Fluorescent labelled specific antiglobulin
11) Second reaction; reaction time on slide 10-15 minutes ← Fluorescent labelled anti goat-anti rabbit globulin
12) 37°C high temperature
13) Phosphoric acid buffer solution pH 8.0 Water wash 10 minutes
14) Phosphoric acid buffer glycerin solution pH 8.0
15) Fluorescent microscope

labelled anti-C. albicans solution, the reactions of various Candida sp. as well as other fungi smear samples were examined. With unadsorbed anti-C. albicans serum or globulin as seen in table 4 in both the direct and indirect methods the C. albicans in the faint blue field of the fluorescent microscope could be observed as glittering, oval, round and rod-shaped blastospore and pseudomycelium and demonstrated a strong fluorescent reaction at the cell wall. Even with other Candida species there was no difference in the reaction, the yellow-green glittering

fluorescence could be observed and the discriminating diagnosis of C. albicans was possible. Although unadsorbed fluorescent labelled C. albicans antibody showed a specificity toward C. albicans antigen, cross-over reactions occurred between the various Candida species and fungi varieties.

Table 4 The Fluorescent Antibody Reaction of Fluorescent Labelled C. albicans Immune Serum With Various Fungi

1) 菌名	2) 株数	3) 蛍光抗体反応(直接法)			
		Heat	Ethanol	Formalin	Acetone
C. albicans	20	+3~+4	+2~+4	+4	+3
C. pseudotropicalis	8	+2~+4	+2~+4	+3	+3
C. guilliermondii	8	+3~+4	+4	+3	+3~+4
C. scotti	4	+2~	+2	+1~+2	+2
C. catenulata	4	+1~+3	+1	+2	+2
C. mycoderma	4	+1~+2	+2	+2	+2
C. pulcherrima	4	+2~	+1	+2	+2
C. krusei	20	+2~+3	+4	+3	+2~+3
C. parakrusei	8	+3	+3	+3~+4	+3
C. stellatoidea	8	+3~+4	+3~+4	+3~+4	+3
C. pelliculosa	4	+2	0	+1	+2
C. pelli	4	+2~+3	+2	+2	+2
C. robusta	4	+2~+3	+1	+2	+1
C. tropicalis	20	+3~+4	+4	+3~+4	+3
C. rosei	4	+2~+3	+3	+3	+3
S. bisporus	4	+2	+1	+2	+2
T. glabrata	4	+3	+3	+3	+3
Cr. neoformans	4	0~+1	0	0	0

4) +4: 輝く黄緑色の蛍光発色を呈するもの
5) +3: 黄緑色の蛍光発色を呈するもの
6) +2: 淡い白黄色の蛍光発色を呈するもの
7) +1: 菌体固有色のみ
8) 0: 菌体固有色のないもの

1) Fungi variety
2) Number of strains
3) Fluorescent Antibody Reaction (Direct Method)
4) + 4: Those with Illuminating Yellow-Green Fluorescence.
5) + 3: Those With Yellow-Green Fluorescence
6) + 2: Those With Thin, pale Yellowish Fluorescence
7) + 1: Only a Characteristic Fungus cell Color
8) 0: Without Characteristic Cell Color

Thus as shown in Table 5, after adsorption with the fluorescent labelled anti C. albicans solution the glittering yellow-green fluorescence arises only with C. albicans (+4), and it was possible to descern clearly the shape of the spore and pseudomycelium. Moreover, there is of course no strong reaction with other Candida sp., S. bisporus, T. glablata, and Cr. neoformans and does not exceed the appearance of a characteristic white cell color (+1). As a characteristic reference, inhibition experiments were conducted and this specificity was clearly seen. From the above mentioned reaction the discrimination of C. albicans from other Candida species and other yeast varieties was possible.

Table 5 Post Adsorption Fluorescent Reactions of Various Fungi

1) 免疫血清	2) 吸収抗原菌株	3) 菌種	4) 株数	5) 蛍光抗体反応 6) 吸収前	7) 吸収後
C. alb.	C. trop.	C. alb.	4	+3~+4	+3~+4
	C. P-L.*	C. trop.	4	+3~+4	+1
		C. stell.	3	+3	
		C. guill.	3	+3	
		C. krusei	3	+3	+1
		C. parakrusei	3	+4	+1
		C. pseudotrop.	3	+3	+1
		T. glabrata	4	+3~+4	
C. guill.	C. alb.	C. alb.	4	+4	
	C. krusei	C. trop.	4	+3~+4	+1
		C. stell.	2	+3	
		C. guill.	3	+3~+4	+3~+4
		C. krusei	3	+3	
		C. parakrusei	3	+4	
		C. pseudotrop.	2	+4	+1
		T. glabrata	3	+4	
C. stell.	S. pastorianus (C. alb.)	C. alb.	3	+3	+1
	C. P-L.*	C. trop.	3	+3	
		C. stell.	3	+4~+3	+3
		C. guill.	2	+4~+3	
		C. krusei	2	+3	
		C. parakrusei	2	+4	
		C. pseudotrop.	2	+3	+1
		T. glabrata	2	+3	+1
T. glab.	C. alb.	C. alb.	2	+3	+1
	S. bispor.	C. trop.	2	+3	+1
	(C. krusei)	C. stell.	2	+3	
		C. guill.	2	+3	
		C. krusei	2	+3	+1
		C. parakrusei	2	+3	
		C. pseudotrop.	2	+3	
		T. glabrata	4	+4	+3~+4

1) Immune serum
2) Adsorbed Heat Treated Antigen
3) Yeast varieties
4) Number of Strains
5) Fluorescent Antibody Reactions
6) Before Adsorption
7) After Adsorption

Identification of C. stellatoidea. The un-adsorbed fluorescent labelled C. stell. solution reacted with all the Candida species and did not show any specificity whatsoever. After adsorption, the fluorescent labelled anti-C. stell. solution was used and examination of this reaction was made in the same manner; however, it did not show a reaction with the other fungi varieties and hetero Candida species, and reacted only with the homo antigen producing a glittering yellow-green fluorescence thus making observation possible. Even with the inhibition experiments, the specificity was evident. Thus after adsorption, the fluorescent labelled anti C. stell. solution could make an identification from the other Candida species and the same results could also be obtained by the indirect method.

C. guill. and T. glablata Identification. With the un-adsorbed fluorescent labelled antibody groups, there were no unique characteristics and mutual cross-over reactions were demonstrated; however, after adsorption the fluorescent labelled C. guill. as well as T. glablata antibody solutions did not show cross-over reactions with respect to antigens, showed individual specificity and observation of the greenish-yellow yeast cell was possible.

Upon observing the fluorescent antibody reactions on smear samples of standard stock cultures of fungi by means of direct and indirect methods after labelling with fluorescent dyes and sufficient adsorption of the sera and γ-globulin solutions specific for C. albicans, C. stell., C. guill. and T. glablata, a specific reaction only with the corresponding antigen was shown by that antibody and it was possible to clearly make distinguishing diagnoses of these Candida species from the others. These results agreed well even with the slide agglutination reactions. Moreover, even on the experimentally mixed smear samples, if C. albicans, C. stell., C. guill. and T. glablata were mixed, by using the corresponding antibody, it was possible to make discriminating diagnoses.

III. RAPID IDENTIFICATION OF VARIOUS FUNGI OBTAINED FROM CLINICAL SOURCES

1. Rapid Identification With The Fluorescent Antibody Reaction of Vaginal Smear Samples

Laboratory tests were made on 96 samples positively identified as fungus. After clean swabbing the exterior and putting on the vaginal scope, the central vaginal wall was swabbed with a sterile cotton swab and five smear samples were prepared. While screening by this method, 24 hour cultures were made on Sabouraud's agar and after a 3-8 hour culture of Sabouraud broth, smear samples were again prepared and identification were made following the aforementioned methods. As comparative tests, determinations were made by means of the Martin-Jones and Lodder-Van Fij methods.

When using the fluorescent antibody method with a layer of anti-C. albicans on the vaginal smears and when C. albicans can be diagnosed,

Table 6 Fluorescent Antibody Method On Clinically Mixed Infectious and Experimentally Mixed Smear Samples

1) 混合菌液	2) 特異抗体	3) 蛍光抗体反応 (間接法)								
		C.alb.	C.kra.	C.trop.	C.stell.	C.guill.	C.p-k.	C.p-t	T. glab.	Cr. neof.
C. alb.+C. krusei	C.alb.	+3+4	+1							
C. alb.+C. trop.		+3+4		+1						
C. alb.+C. stell.		+3			+1					
C. alb.+C. guill.		+3+4								
C. alb.+C. p-k.*		+3+4					+1	+1		
C. alb.+C. p-t.**		+3								
C. alb.+T. glab.		+3								
C. alb.+C. trop.+C. stell.		+3		+1	+1					
C. guill.+C. alb.	C. guill.	+1				+3				
C. guill.+C. trop.				+1		+3				
C. stell.+C. alb.	C. stell.	+1			+3					
C. stell.+C. trop.				+1	+3					
T. glab.+C. alb.	T. glab.	+1							+3+4	
T. glab.+C. trop.				+1					+3	
Cr. neofor.+C. alb.	Cr. neofor.									+3
Cr. neofor.+C. trop.										+3

*: C. parakrusei **: C. pseudotropicalis.

1) Yeast mixture
2) Specific Antibody
3) Fluorescent Antibody reaction (Indirect Method)

the glittering greenish-yellow fluorescence of round or oval Candida spores could be seen. The walls fluoresced strongly while the central portion only weakly. Although the nuclei of vaginal epithelial cells presents a weak greenish-yellow color, with adsorbed fluorescent antibody solution prepared using mouse acetone-treated powder the nonspecific reaction decreases and the differentiation of fungi could be clearly made. However the complete elimination was not possible.

From the vaginal smear samples C. albicans was detected in 65 of 96 samples or in 67.7% and neither C. stell. nor C. guill. could be detected even in a single sample. T. glabata was detected in 21 of 96 samples or 21.8%. The others undetectable were 10 in number (10.5%) that is to say, these were other than C. albicans, C. guill., C. stell. and T. glabata. Comparing the present results to results of detection depending on

biological characteristics and from serological methods, Table 7 reveals that for C. albicans the fluorescent antibody method was successful with 65 samples, the serological detection method with 61 samples and the biological method with 68 samples. The results of the fluorescent antibody method compared to the biological detection method was in a ratio of 1:0.9. The detection of T. glabata from each of the three methods was 21 samples (21.8%) and the results were in complete agreement.

Table 7 Comparison of Fluorescent Antibody Method To Other Methods on Standard and Clinical Stocks

1)	2)	3)	4)	5)
6)				
C. alb.	15	15 (100%)	13 (86.6%)	15 (100%)
C. stell.	5	5 (100%)	5 (100%)	5 (100%)
C. guil.	5	5 (100%)	5 (100%)	5 (100%)
T. glab.	5	5 (100%)	4 (80%)	5 (100%)
7)	9)			
	96	C. alb. 65 (67.7)	C. alb. 61 (63.4)	C. alb. 65 (70.8)
		C. stell. 0	C. stell. 0	C. stell. 0
		C. guil. 0	C. guil. 0	C. guil. 0
		T. glab. 21 (21.8)	T. glab. 21 (21.8)	T. glab. 21 (21.8)
	10)	その他 10 (10.5)	10) その他 14 (14.5)	10) その他 7 (7.4)
8)				
	30	C. alb. 29 (96.6)	C. alb. 28 (93.4)	C. alb. 28 (93.3)
		C. stell. 0	C. stell. 0	C. stell. 0
		C. guil. 0	C. guil. 0	C. guil. 0
		T. glab. 1 (3.4)	T. glab. 1 (3.3)	T. glab. 1 (3.3)
	10)	その他 0	10) その他 1 (3.3)	10) その他 1 (3.3)

1) Source
2) Number of strains
3) Identification results according to fluorescent antibody reaction
4) Identification results according to serological method
5) Identification results according to biological method
6) Standard stock strain
7) Strain from vaginal separation
8) Strain separated from oral cavity of newborn
9) Clinical strain
10) Others

2. Rapid Identification of Fungus on Smear Samples From The Mouths of Newborn Infants

Smear samples were prepared from thrush of newborn infants and fungi from the oral cavity using the indirect method. From the fluorescent antibody reactions of 30 samples from the oral cavity, C. albicans was identified in 29 samples as shown in Table 8. On the other one sample

Table 8 Results from Methods Other Than Smear Samples Compared with Fluorescent Labelled C. albicans Anti globulin

1)	2)	3)	4)
5)	54(79.4%)	62(91.2%)	65(95.6%) / 68 例 6)
7)	8(27.5)	20(68.9)	29(100) / 29 例 8)
9) T. glabrata			
10)	11 (52.4)	18 (85.7)	21(100) / 21 例 11)
12)	0	1 (100)	1(100) / 1 例 13)

1) Origin
2) Cotton swab
3) Broth
4) Broth sediment
5) Vaginal
6) Samples
7) Oral cavity of newborn infants
8) Samples
9) Fluorescent labelled anti T. glablata globulin
10) Vaginal
11) Samples
12) Oral cavity newborn
13) Sample

the specific anti T. glablata antibody was used and only the specific reaction shown by T. glablata was evident.

Beside the preparation of smear samples, that is to say, either by the direct cotton swab method or from eight hour Sabouraud's broth cultures, on the smear samples of the sediments a layer of specific anti-C. albicans and T. glablata sera were used. The results obtained from fluorescent antibody method are comparatively examined in Table 8. From 68 vaginal Candida samples, identification was made in 54 samples (79.4%) by the direct cotton swab method, in 62 (91.2%) of the smear samples after short broth culture and in 65 (95.6%) of the smear samples from the broth sediments. In the 30 samples positive for fungi from the oral cavities of newborn there were 8 samples (27.5%) out of 29 samples by the direct

cotton swab method and in 20 samples (68.9%) after broth culture. In the sediment samples the fungi were detected in all 29 samples.

SUMMARY

The use of rapid diagnosis of antigen on smear samples and of distribution determinations have been made with virus [21], Toxoplasma [24], yeast-like fungi [39], Streptococcus [27], Staphylococcus and Escherichia coli [29] and the useful significance has been widely reported. Here, the rapid diagnosis of fungus infection from smear samples was used for routine identification and if the improvement can be applied to previous procedures, one wonders whether this does not greatly affect epidemiology and the diagnosis of the nature of infections.

Gordan (1958) [39] attempted a differentiating diagnosis of C. albicans from other disease-causing Candida species using the fluorescent labelled anti-C. albicans solution and although adsorption was accomplished, partial non-specific reactions were shown and C. albicans, C.trop. as well as C. stell. could not be differentiated. In 1958 he was able to distinguished between C. albicans and C. stell. [40], while in 1960 Kaplan and Kaufman [41] using specific anti-C. albicans solution were successful in the differentiating diagnosis.

The result of testing this reaction on the various Candida species with the unadsorbed anti-Candida serum was similar to the non-specificity (cross-over reaction) of Gordan's results and it was not possible to specifically dye the antigens corresponding to each antibody. However, in the rapid diagnostic method for fungal infections various simple diagnostic media have been devised [42 - 44] and considerable verifying results have been presented, but these do not exhibit a foundation of specificity whatsoever, and on the point that they are not reliable, using the aforementioned unadsorbed fluorescent labelled anti-Candida serum with the Candida smear samples taken together, a rapid diagnosis was possible.

Again on the matter of eliminating the nonspecific factor of the unadsorbed fluorescent labelled Candida serum, there was considerable difficulty and there were instances where complete elimination was not accomplished. In those cases, it is probably imperative to exchange the adsorbing fungi and to change to other immune rabbit serum instead of simply repeating the adsorption haphazardly. After succeeding in obtaining the specific immune sera corresponding to the pathogenic Candida species, C. albicans, C. stell., C. guill. and T. glab., it became possible to carry out discriminating diagnoses of antigens corresponding to the antisera.

With respect to clinical research, diagnosis of fungi was made on smear samples by the cotton swab method directly from the site of infection. In the microscopic examination with the Gram stain difficulties are often encountered in the diagnosis and the present method makes a simple

diagnosis possible from the standpoint of materials which do not give rise to antigen-antibody reactions according to the specificity of reaction making examination impossible. However in the diagnosis of specific reactions it is necessary to use as contrast a foreign antiserum as well as a common domestic rabbit serum and then carry out the diagnosis. Moreover, in the present method there are non-specific reactions present; however, using acetone-treated visceral powder, it is a matter of eliminating fairly well the reactions with respect to decidual cells, structural cells and corpuscles.

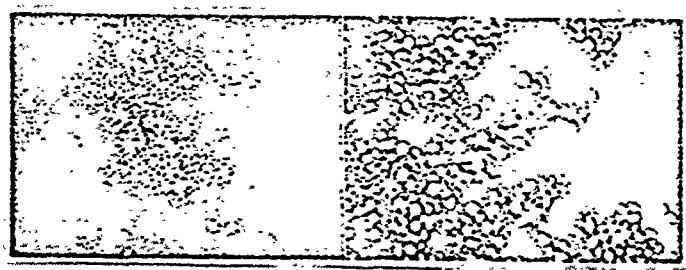
Especially in thediagnosis of fungi in smear samples from the direct cotton swab method, due to the problem of the fungi as well as the non specific reactions of decidual cells, blood cells and structural cells, the ratio ofpositive identifications has a tendency to fall. However, smear samples after a short period of broth culture, show good results and are also advantageous for diagnosis.

Not only whether this is adequately useful in the rapid diagnosis of fungus infections in obstetrics as above should be considered but also its significance in epidemiology.

(left)
Photograph 1 Fluorescence picture of Candida albicans. Spores due to fluorescence labelled specific anti C. albicans (40 X 10).

(right)
Photograph 2 Fluorescence of standard stock Candida albicans. Pseudomycelium and spore due to fluorescence labelled specific anti-C. albicans

(1) (2)



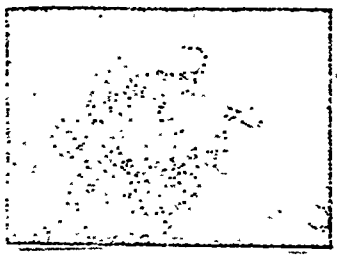
(3) (4)

(1) Photograph 3. Candida albicans from vaginal smear samples. Pseudomycelium and spore due to fluorescent labelled specific anti-C. albicans serum.

(2) Photograph 4. Discriminating diagnosis of C. albicans from smear samples of experimentally mixed C. albicans and C. tropicalis (40X10). Identification of C. albicans from fluorescent labelled anti-C. albicans serum. (Spores emanating fluorescence are C. albicans).

(3) Photograph 5. Discriminating diagnosis of C. albicans from smear sample of experimentally mixed C. albicans and Cr. neoformans (40X10). Identification of C. albicans by means offluorescent labelled anti-C. albicans serum.

(4) Photograph 6. Spore from Cr. neoformans fluorescent labelled specific anti-Cr. neoformans.



Photograph 7. Discriminating diagnosis of C. albicans from experimentally meixed smear samples of C. albicans and C. tropicalis (40 X 10). Identification of C. albicans by means of fluorescent labelled anti- C. albicans serum. (Spores emanating fluorescence are C. albicans).

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