Clinical application of a rapid and simple supersensitive identification method for the identification of Oral Candida species from oral rinse specimens

Department of Oral surgery and Oral Medicine,
Kagoshima University Hospital
KAGOSHIMA JAPAN
Yoshiaki KAMIKAWA, T.NAGAYAMA, R. SAKAMOTO, K. SUGIHARA

Materials and Methods

Seventy cases of oral candidiasis patients who were diagnosed and treated in the Department of Oral Medicine of Kagoshima University Hospital were investigated and their results of our new candida test using the PCR (polymerase chain reaction) method and the conventional old culture method were thus compared. Candida DNA were extracted from FTA Classic Card（Whatman ®）impregnating oral rise specimens. In addition, PCR amplification was then performed using a modified method of KAMBE with the addition of Ampdirect (SIMADZ ®). The products were then electrophoresed.

Abstraction DNA from *Candida species* and PCR

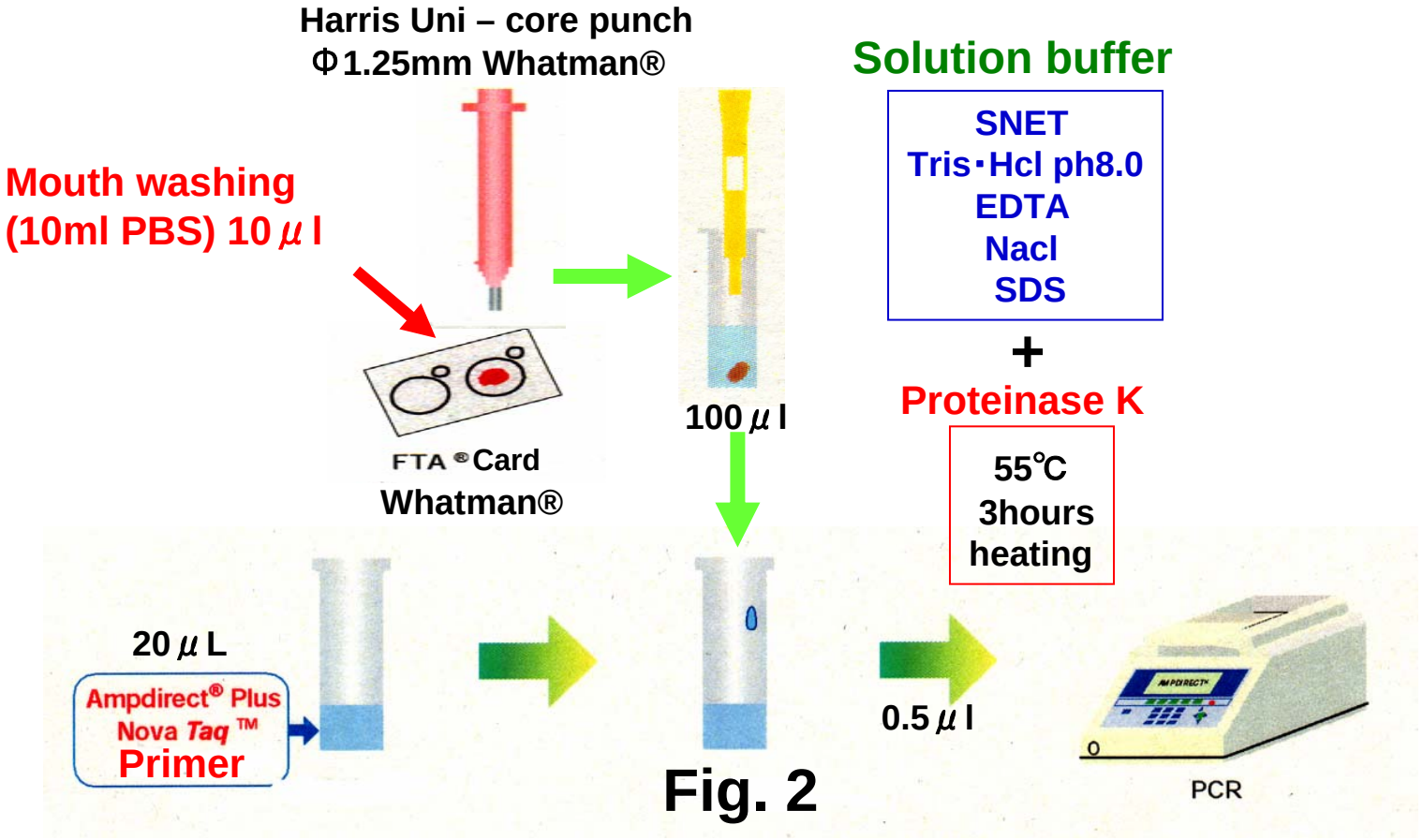


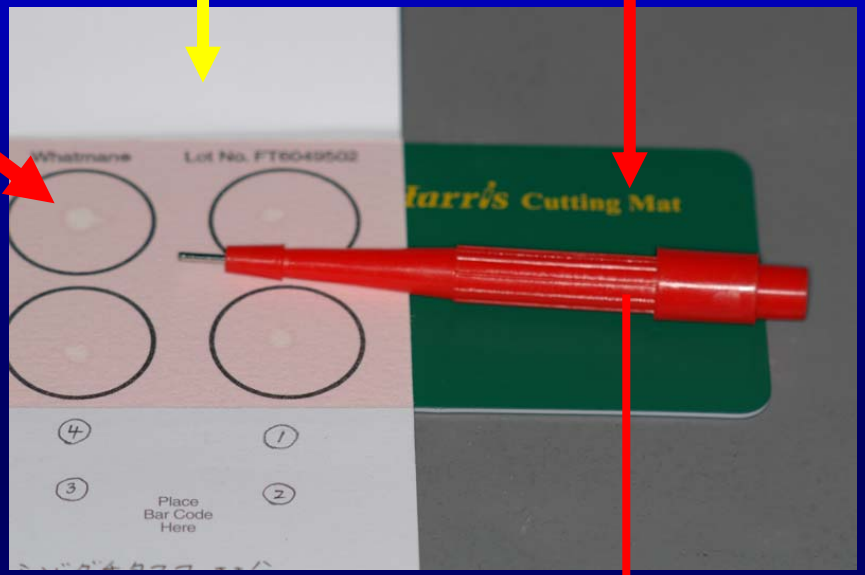
Fig. 2

Fig. 3

FTA - Card
Whatman®

Harris Uni – core
Φ1.25mm Whatman®

Mouth washing
(10ml PBS) 10μl



Solution buffer

	SNET
Tris・Hcl ph8.0	20mM
EDTA	5mM
Nacl	400mM
SDS	0.3%

+ Proteinase K= 100 μL

Punched out and put into solution buffer

Heated 55℃
three ours

PCR

Fig. 6

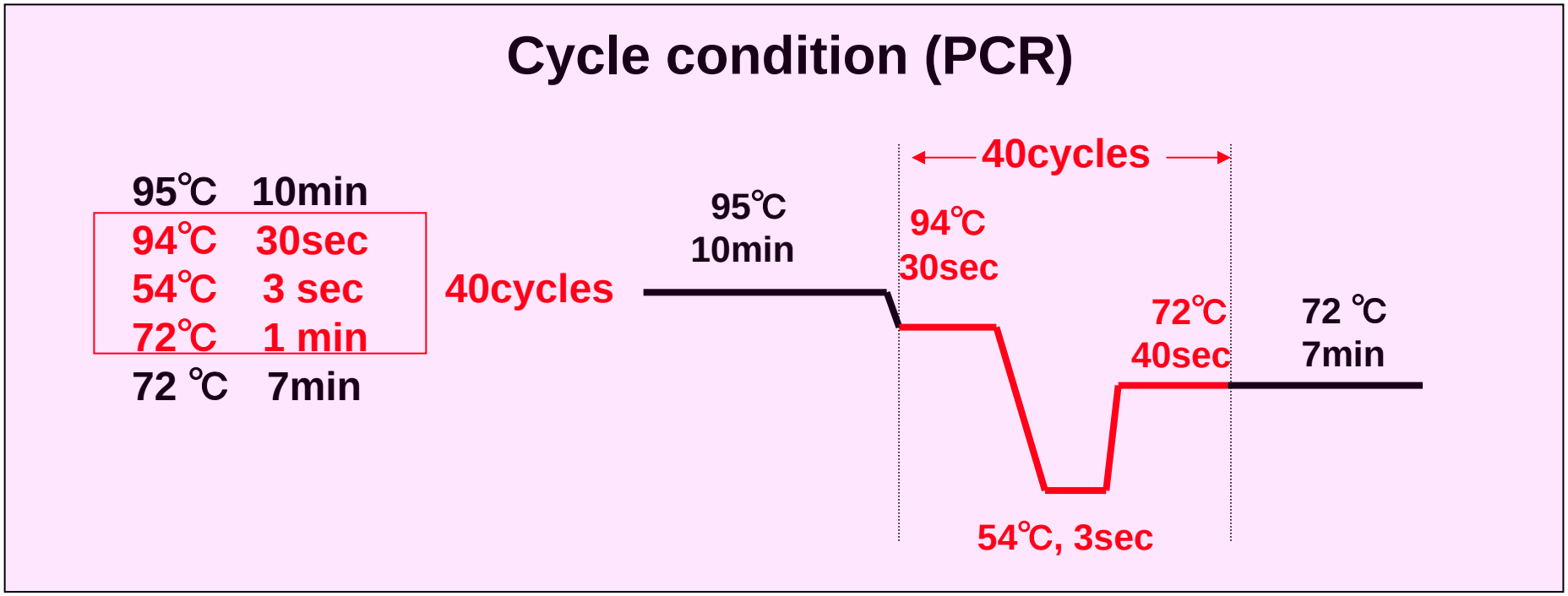
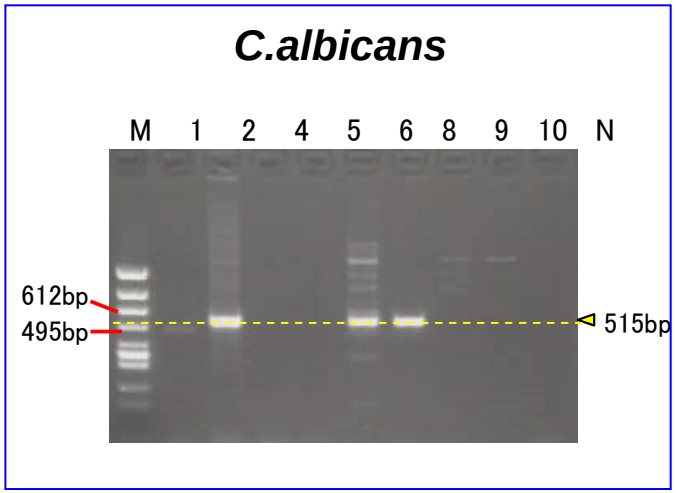
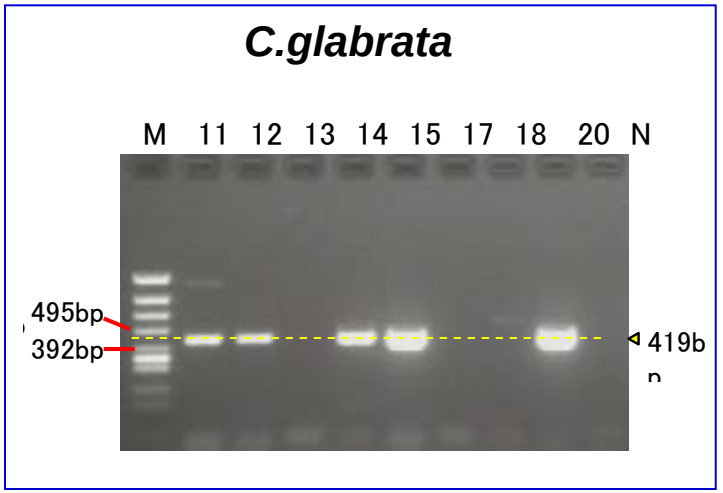
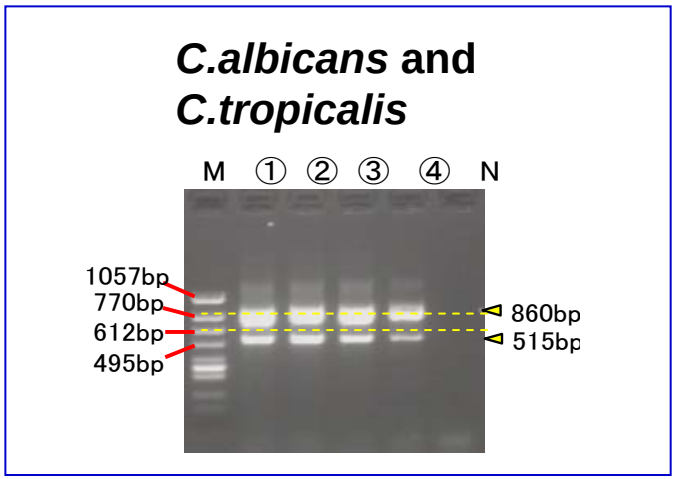
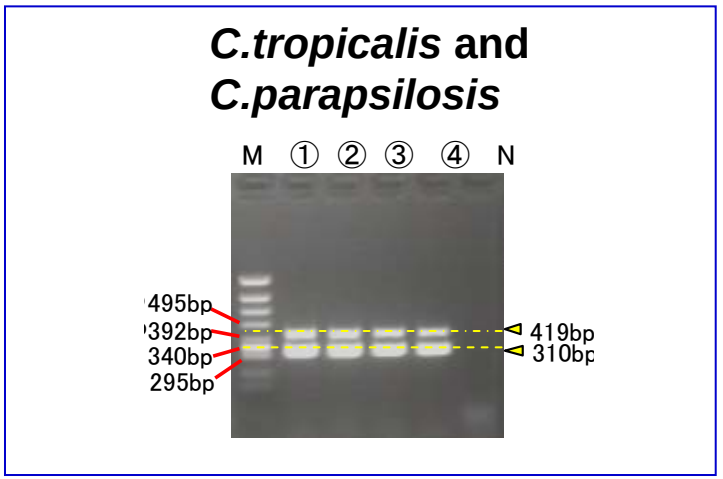

Cycle condition (PCR)
95℃ 10min
94℃ 30sec
54℃ 3 sec
72℃ 1 min
72 ℃ 7min
40cycles
95℃
10min
40cycles
94℃
30sec
72℃
40sec
72 ℃
7min
54℃, 3sec

Fig. 7









Candida species	Objective product(bp)
C.albicans	515
C.tropicalis Ⅰ	318
C.tropicarls Ⅱ	860
C.parapsilosis Ⅰ	837
C.parapsilosis Ⅱ	310
C.glabrata	419

M : ΦX174*Hinc* Ⅱ digesta
N : No template

Using this new method we were able to detect the kinds of *Candida species* from all the case of oral rinse which were also found to be culture positive based on conventional culture methods.