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

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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

Mouth washing (10ml PBS) 10 μl

Solution buffer

- SNET
- Tris·Hcl ph8.0
- EDTA
- NaCl
- SDS

+ Proteinase K

55°C
3 hours heating

Fig. 2
Mouth washing (10ml PBS) 10 μl

Solution buffer

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tris·HCl ph8.0</td>
<td>20 mM</td>
</tr>
<tr>
<td>EDTA</td>
<td>5 mM</td>
</tr>
<tr>
<td>NaCl</td>
<td>400 mM</td>
</tr>
<tr>
<td>SDS</td>
<td>0.3%</td>
</tr>
<tr>
<td>Proteinase K</td>
<td>100 μL</td>
</tr>
</tbody>
</table>

Heated 55°C three ours

Punched out and put into solution buffer

PCR
Cycle condition (PCR)

95°C 10min
94°C 30sec
54°C 3 sec
72°C 1 min
72 °C 7min

40cycles

95°C 10min
94°C 30sec
54°C, 3 sec
72°C 40sec
72 °C 7min

40cycles

Fig. 6
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.