Brief report

Genetic confirmation of Entamoeba histolytica by the real-time polymerase chain reaction (rPCR) with formalin-fixed paraffin-embedded specimen (FFPE) of colorectal biopsy

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Background: Entamoeba histolytica should be rapidly confirmed by genetic analysis. In this report we could establish the rapid and convenient rPCR-FFPE method and reported our case.

Case report: 51-year-old male patient revealed rectal macrophages phagocytizing amebas, which were confirmed as Entamoeba histolytica by both the usual PCR-FFPE method followed by gel electrophoresis and fluorescence reaction (usual PCR-FFPE method) and the rPCR-FFPE method.

Conclusion: The rPCR-FFPE method provided a definitive diagnosis of Entamoeba histolytica more readily than the usual PCR method.

Key words: Entamoeba histolytica, genetic analysis, the real-time polymerase chain reaction with formalin-fixed paraffin-embedded specimen (rPCR-FFPE method), usual PCR method followed by gel electrophoresis and fluorescence reaction (usual PCR-FFPE method)

Discussion

The usual PCR-FFPE method required quite a complicated procedures and lot of works. In contrast, the rPCR-FFPE method could shave time off and was more convenient method to get the final diagnosis than the usual PCR-FFPE method.

In the rPCR-FFPE method, the preferable annealing temperatures are 65°C for pathogenic ameba and 60°C for non-pathogenic ameba. The single screening test for both pathogenic amebas and non-pathogenic ones could be used under the annealing temperature at 60°C and differentiated by the dissociation curve with their different peak temperatures.
Reference


Figure 1. Chart of dissociation curve in the rPCR-FFPE method under the annealing temperature at 60°C.

Two peaks at 77°C of specific peak and 82°C of small non-specific one in pathogenic ameba. Single peak at 72°C in non-pathognetic ameba.
circle: PCR using primers for pathogenetic ameba, square: PCR using primers for non-pathoggetic ameba, x-axis: temperature (°C), y-axis: fluorescent degree.
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Figure 2. Chart of dissociation curve in the rPCR-FFPE method under the annealing temperature at 65°C.
Single peak at 77°C of specific peak and a small negligible non-specific hump in pathogenetic ameba. circle: PCR using primers for pathogenetic ameba, x-axis: temperature (°C), y-axis: fluorescent degree.

<table>
<thead>
<tr>
<th></th>
<th>usual PCR-FFPE</th>
<th>rPCR-FFPE</th>
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<tbody>
<tr>
<td>denaturation</td>
<td>94°C 10’</td>
<td>denaturation 94°C 10’</td>
</tr>
<tr>
<td>3 step PCR</td>
<td>94°C 1’</td>
<td>2 step PCR 95°C 5’</td>
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<tr>
<td>denaturation</td>
<td>94°C 1’</td>
<td>2 step PCR 95°C 5’</td>
</tr>
<tr>
<td>annealing</td>
<td>60–68°C 20’</td>
<td>annealing 60–68°C 20’</td>
</tr>
<tr>
<td>elongation</td>
<td>35 cycles</td>
<td>cycles 40</td>
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Table 1. Procedure of PCR

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