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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)

Background

Infectious enterocolitis by Entamoeba histolytica is pathologically diagnosed by the confirmation of macrophages phagocytizing amebas, which is often difficult to find microscopically because of the interruption-driven environment with marked inflammatory debris and degeneration. More accurate diagnostic tool has been required pathologically. The PCR-FFPE method of colorectal lesions was tried to detect Entamoeba histolitica, and we, furthermore, compared its superiority between the usual PCR-FFPE method and the rPCR-FFPE method in this report.

Case report

51-year-old male patient complained of diarrhea and rectal erosions were identified during endoscopy. Several macrophages were histologically found in necrotic debris on the surface of rectal epithelium. The intracytoplasmic inclusions suggested amebas microscopically. The FFPE specimen was routinely processed with two types of primers ; pathogenetic pairs and non-pathogenetic ones (1, 3). Both the usual PCR-FFPE method and the rPCR-FFPE method were done, the latter of which was done with real-time PCR : Mx3000P Real-Time QPCR system (Agilent) and SYBR™ Green I method, SYBR™ Premix EX Taq™ (Tli RNaseH Plus) RP420A (Takara) (Table 1). The usual PCR-FFPE process required 11 hours to get the expected bands at 100 base-pair. The rPCR-FFPE method against pathogenetic ameba under the annealing temperature at 60℃ showed the specific peak at 77℃ with a small non-specific peak on the chart of dissociation curve within the total curse of 4 hours. The latter non-specific peak could be reduced to avoid the false-positive peak under the annealing temperature at 65℃. No peak of pathogenetic ameba could be found under the annealing temperature over 65℃. Non-pathogenetic amebas showed a single peak at 72℃ under the annealing temperature at 60℃ and no peak under the annealing temperature over 60℃ (Figure 1, 2).

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℃ for pathogenetic ameba and 60℃ for non-pathogenetic ameba. The single screening test for both pathogenetic amebas and non-pathogenetic ones could be used under the annealing temperature at 60℃ and differentiated by the dissociation curve with their different peak temperatures.

Referrence

1．Ikarashi, T et al. Detection of monoclonality in B- and T-cell lymphoma by the use of polymerase chain reaction of formalin-fixed paraffin-embedded tissues. Niigata-ken Koseiren Med J 2000 ; 10 : 10－5.
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和　文　抄　録

短報

結腸直腸生検のホルマリン固定パラフィン包埋材料（FFPE）を使ったリアルタイム・ポリメラーゼ連鎖反応（rPCR）による赤痢アメーバの遺伝子学的同定

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背景：赤痢アメーバを rPCR-FFPE によって迅速に同定することができたので、1 症例を報告した。

症例報告：51歳の男性患者は、直腸生検材料においてアメーバを貪食した大食細胞を示し、ゲル電気泳動・蛍光反応による従来の PCR-FFPE 検査と rPCR-FFPE 検査において赤痢アメーバを同定できた。

結論：rPCR-FFPE 検査は、従来の PCR 検査に比較して、容易に赤痢アメーバを同定できる。

キーワード：赤痢アメーバ、遺伝子分析、ホルマリン固定パラフィン包埋標本を使ったリアルタイム・ポリメラーゼ連鎖反応検査（rPCR-FFPE）、ゲル電気泳動・蛍光反応による従来の PCR 検査

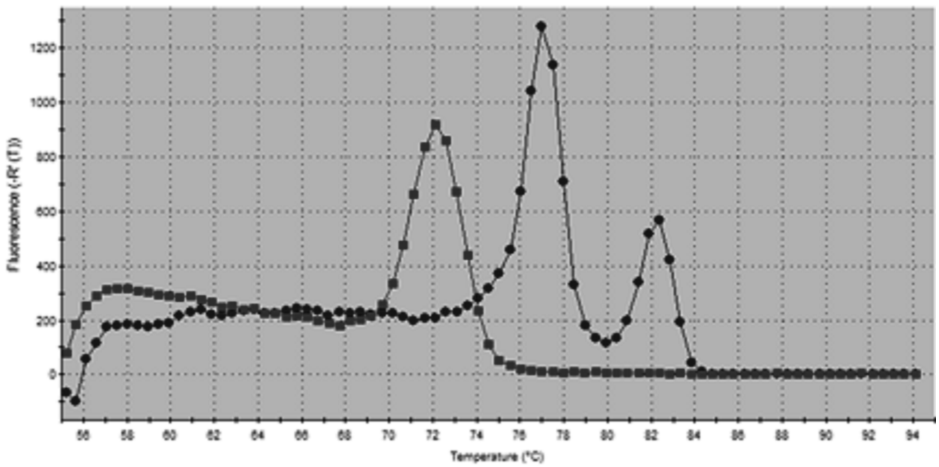

Figure 1. Chart of dissociation curve in the rPCR-FFPE method under the annealing temperature at60℃..
Two peaks at 77℃ of specific peak and 82℃ of small non-specific one in pathogenetic ameba. Single peak at 72℃ in non-pathogenetic ameba.
circle : PCR using primers for pathogenetic ameba, square : PCR using primers for non-pathogenetic ameba, x-axis : temperature（℃）, y-axis : fluorescent degree.

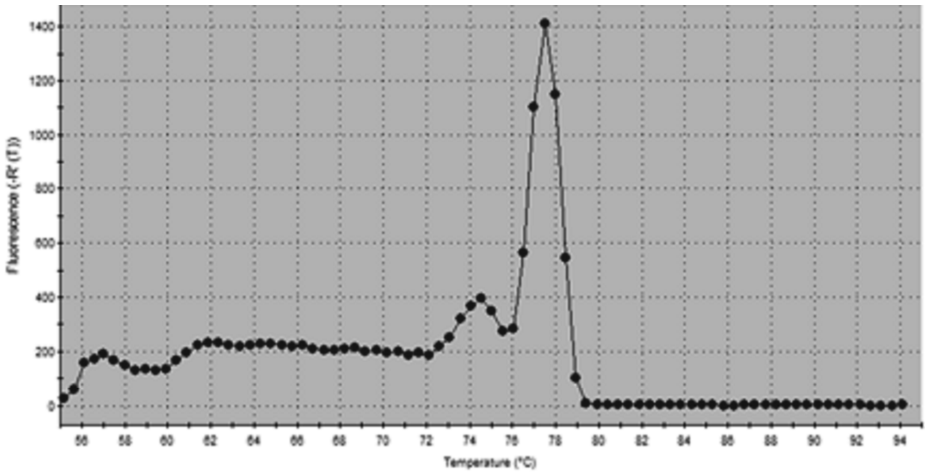

Figure 2. Chart of dissociation curve in the rPCR-FFPE method under the annealing temperature at 65℃..
Single peak at77℃of specific peak and a small negligible non-specific hump in pathogenetic ameba.
circle : PCR using primers for pathogenetic ameba, x-axis : temperature（℃）, y-axis : fluorescent degree.

Table 1. Procedure of PCR

usual PCR-FFPE			rPCR-FFPE		
denaturation	94℃	10'	denaturation	94℃	10'
3 step PCR			2 step PCR		
denaturation	94℃	1'		95℃	5″
annealing		1'30″		60-68℃	20″
elongation		1'30″			
cycles		35		cycles	40

（2016／06／23受付）