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Report

Trial of rapid diagnosis of ifection and individual confirmation against tissue contamination by real time PCR（polymerase chain reaction）of SYBR Green method to paraffin-embedding specimens

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Objective : We have provided a pathological diagnosis on the basis of genetic PCR analysis using paraffine-embedded materials for 10 years. For labor saving and more precision of results, we tried to use the real time PCR.

Materials : ① paraffin-embedded block（＃15-12522）, EBV（Ebstein-Barr virus）infection, ② mixed-up cases（＃15-12123 and ＃15-12124）, multiple sampling from breasts, examined three kinds of STR（short tandem repeat）regions of D3S1358 on chromosome 3, D5S818 on chromosome 5, and vWA on chromosome 12(2)(Tab. 1).

Method : The nucleic acid extraction process was reported in previous papers（1, 2）; the real-time PCR was done using Mx3000P Real-time QPCR System（Agilent Technologies）and SYBR® Premix Ex Taq ™（Tli RNaseH Plus）（Takara）.

Results : ① In real time PCR of EBV infection, the positivity required 29-30 cycles of PCR, and over 33 cycles led to false-positive result. In dissociation curve, the peak of positive case was 85℃（Fig. 1）. ② About the correspondence at the mix-up of specimens, we were able to identify its confusion by the STR mutation of vWA on an electrophoretic gel, which was also confirmed as a peak temperature difference of the dissociation curve in real time PCR（Fig. 2, 3）.

Discussion : We were able to shorten the time required of 1-2 days for amplification / electrophoresis / confirmation of band in the PCR process to only two hours by automation process with the real-time PCR. The identification methods became the digital confirmation from visual confirmation, and the precision of judgment was able to improve, too.
There were several false-positive cases in the real-time PCR diagnosis in lymphoma, tuberculosis, and tsutsugamushi disease diagnosed by the conventional nested PCR, which prevented from practical use at present.

Key words : genetic analysis for pathological diagnosis, real time PCR（polymerase chain reaction）, efficiency, accuracy, paraffine-embedded pathological specimen, EBV infection(Epstein-Barr virus), contamination of other person's tissue, short base tandem repeat sequence (Short tandem repeat : STR), internal variability of STR, D3S1358 on chromosome 3, D5S818 on chromosome 5, vWA on chromosome 12, genetic individual identification

Reference

1．Hidehiro Hasegawa, Takemitsu Katagiri, Toshihiko Ikarashi. Detection of Epstein-Barr virus（EBV）DNA and RNA from gastric carcinoma with lymphoid stroma by genetic analysis. Niigata-Ken Koseiren Med J 2006 ; 15 : 65-9.

2．Hidehiro Hasegawa, Toshihiko Ikarashi. Genetic individual identification of paraffin-embedded pathological preparations to rule out the sporadic contamination of other patient's tissue during preparation of paraffin sections of pathological examination. Niigata-Ken Koseiren Med J 2011 ; 20 : 46-9.

和　文　抄　録

報告

SYBR Green 法リアルタイム PCR 導入によるパラフィン包埋材料を使った PCR 遺伝子診断の効率化の試み
―感染症診断、検体取り違い診断の実用化―

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目的：従来我々はパラファイン包埋材料を使って PCR 診断を実施してきたが、リアルタイム PCR による省力化とその精度を確認し実用化したので報告する。

材料：①　＃15-12522EBV 感染症、②　＃15-12123, ＃15－12124　二症例間の検体取り違え事例に関して検討した。②に関しては、12番染色体上に存在する vWA、5番染色体上の D5S818と3番染色体上の D3S1358の3種類の STR 領域を検討した。

方法：核酸抽出工程は従来方式とし、リアルタイム PCR は Mx3000P Real-time QPCR System（Agilent Technologies）、SYBR® Premix Ex Taq™（Tli RNaseH Plus）（タカラ）を使用した。

結果：①　EBV 感染症に関しては、増幅において、PCR29－30サイクルで陽性となり33サイクル以上で偽陽性となる。解離曲線において、陽性例ピークは85℃であった。②　検体混入時の対応に関しては、STR 検索において従来式電気泳動上確認できた vWA の変異をリアルタイム PCR においては解離温度のピーク差として同定できた。

考察：従来 PCR 工程の増幅・電気泳動・バンド確認に要する1～2日間の所要時間を、リアルタイム PCR による自動化により2時間に短縮できた。結果の判定も目視確認からデジタル確認となり精度を向上できた。従来の nested　PCR による遺伝子診断を実施していたリンパ腫、結核、ツツガムシに関しては、リアルタイム PCR 診断においては偽陽性症例があり現時点では実用化に至らなかった。

キーワード：病理診断精度を担保する遺伝子診断、リアルタイムポリメラーゼ連鎖反応（PCR）、有効性、正確性、パラフィン包埋検体、EBV 感染症、検体混入・取り違え、遺伝学的個人識別、短鎖直列型反復配列（STR）変異、染色体3番 D3S1358、染色体5番 D5S818、染色体12番 vWA

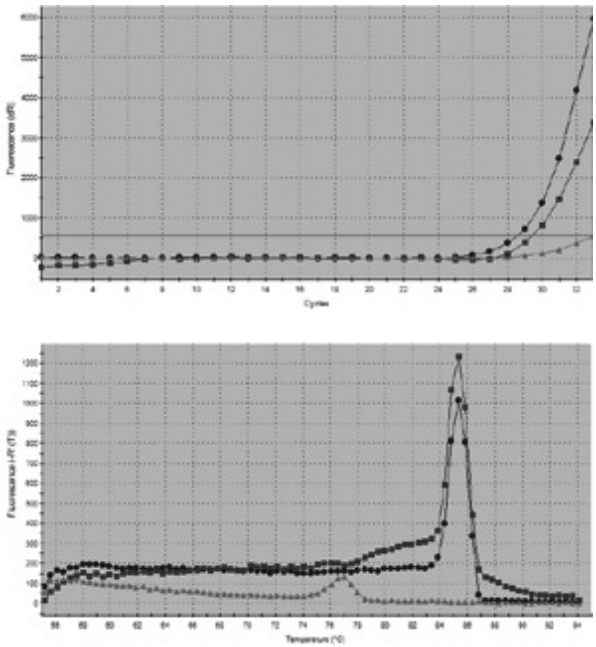

Fig 1. Real time PCR of EBV.
Upper : Amplification plots showed positive PCR. Bottom : Dissociation curve was positive to EBV with the same peak as positive control.
Circle : sample, square : positive control, triangle : negative control, PCR : polymerase chain reaction, EBV : Epstein-Barr virus.

Table 1. Clinical, pathological, and genetic list of two cases with mutual intermixed specimens

Number of histological examination			H15-12123		H15-12124
			#1, 2, 4, 5	#3	#1, 2
Clinical diagnosis			R, D, 1.6x1cm, benign		L, C, 1cm, malignant
Blood type			A		A
Finding in hematoxylin-eosin stain			benign	malignant	
PCR	electrophoresis	vWA	Two bands or single lower broad band	Single upper broad band	
		D3S1358	No significance		
		D5S818	No significance		
	Real time: dissociation curve ℃	vWA	78.5℃	77.5℃	
		D3S1358	No significance		
		D5S818	No significance		

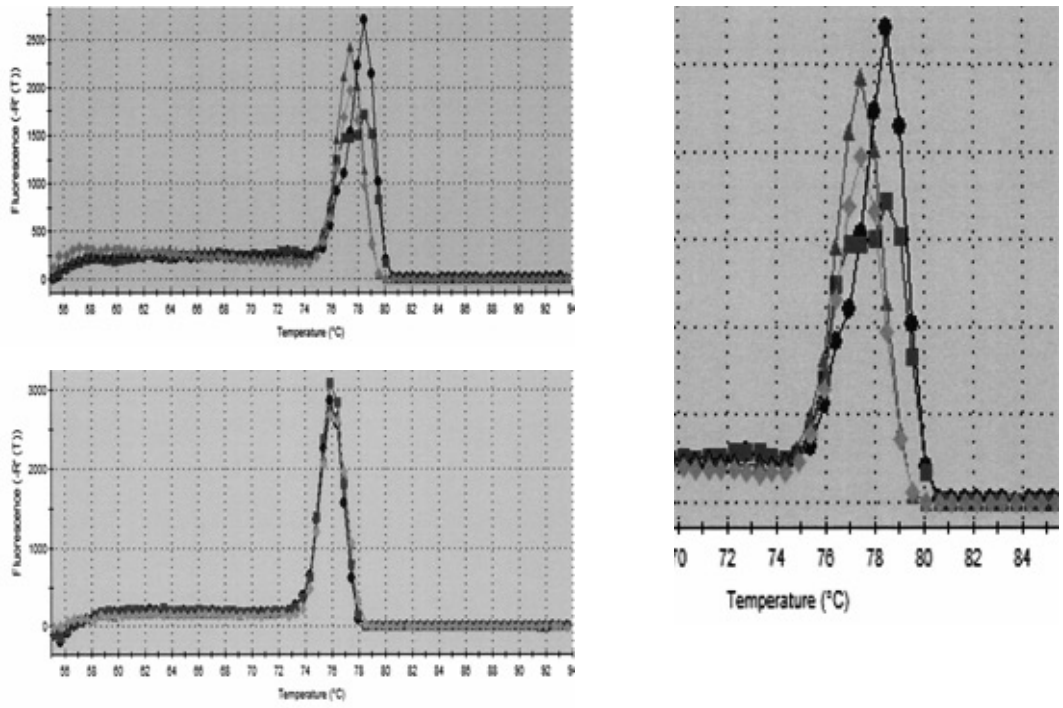

Fig 2. Dissociation curve of real time PCR of vWA and D5S818.
Upper : vWA showed two groups of different peak, samples of #15-12123-1, 2 at 78.5℃, and samples of #15-12123-3 and #15-12124-1 at 77.5℃, respectively. Middle : enlarged view of upper figure. Bottom : D5S818 showed same peak of 76℃.
Circle : sample #15-12123-1, square : sample #15-12123-2, diamond : sample #15-12123-3, triangle : sample #15-12124-2, PCR : polymerase chain reaction, EBV : Epstein-Barr virus.

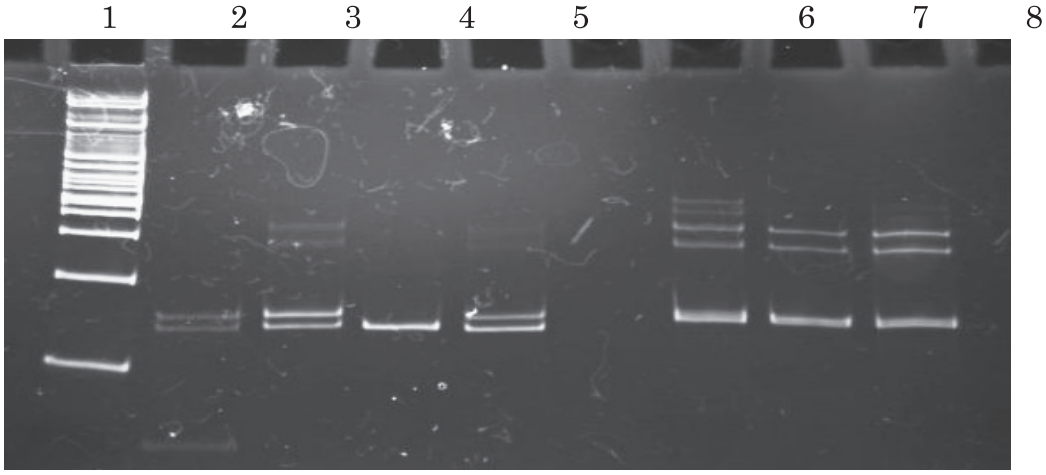

Fig 3. Electrophoresis of PCR of vWA.
Lane 2-5：benign lesions showed 2 bands, 138-162 base-pairs. Lane 6-8 : malignant lesions showed close bands looked alike a single upper broad band.
Lane 1 : 100-base-pair marker, lane 2-4 : ＃15-12124-1, 2, 4, 5 of benign lesions, lane 6-8：＃15-12124-1, 2, and＃15-12123- 3 of malignant lesions. PCR : polymerase chain reaction.

（2016／01／10受付）