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Original Paper

Michel's Transport Medium as an Alternative to Liquid Nitrogen for PCR Analysis of Skin Biopsy Specimens

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Key Words
Michel's medium · PCR · Skin biopsy · DNA

Abstract
Formalin fixation and paraffin embedding are standard procedures for histopathological diagnosis and allow long-term archiving of tissue specimens. The cross-linking properties of formalin cause fragmentation of nucleic acids and reduce the sensitivity of PCR analysis. Michel's medium is a well-established transport medium used by dermatologists for biopsy transport to maintain tissue-fixed immunoreactants prior to direct immunofluorescence and immunoelectron microscopy. Here we report that Michel's medium also allows short-term preservation of DNA for PCR analysis and permits amplification of amplicons larger than 1 kb. Therefore, Michel's medium appears to be a reserve medium for performing PCR when no other samples are available.

Introduction

Formalin fixation and paraffin embedding (FFPE) are standard procedures for histopathological diagnosis and allow long-term archiving of tissue specimens. Unfortunately, the cross-linking properties of formalin cause fragmentation of nucleic acids and reduce the quality of DNA extracted from FFPE, which may result in low sensitivity of PCR tests in some samples [1]. Thus, the gold standard for molecular analysis remains fresh-frozen tissue,

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which is not convenient for specimen conservation and transportation. In dermatology, an alternative conservation and transport medium commonly used is Michel's transport medium. This medium, initially described by Michel et al. [2] in 1972, has been simplified by Niedecken and Lange [3] and is currently used by most dermatologists for biopsy transport to laboratories for the detection of tissue-bound immunoreactants by direct immunofluorescence microscopy (DIF) [4–6]. More recently, normal saline was shown to be an alternative to Michel's transport medium for DIF studies, but it can only be used if the specimens are transported to the laboratory within 24 h [7].

We wanted to assess whether DNA extracted from biopsies made for DIF and transported in various transport media including Michel's transport medium was equally suited for molecular testing by PCR.

Materials and Methods

Tissue Samples

All samples were from the dermatology laboratory at the Geneva University Hospital (table 1). Twelve biopsies in Michel's medium (55 g NH_4SO_4 per 100 ml 0.9% NaCl, pH 7.4) [3] and 7 biopsies in 0.9% NaCl were sent for DIF by dermatologists outside of the Hospital. Specimens in Michel's medium and in 0.9% NaCl were sent by regular mail and arrived at the laboratory within 3 days after sending. These specimens were rinsed in PBS for 3 × 10 min and frozen at –20°C. Twelve biopsies were from patients examined at the dermatology clinic, Geneva University Hospital. Specimens were transferred to cryotubes, snap-frozen in liquid nitrogen and transported in liquid nitrogen to the laboratory and immediately stored at –20°C. All specimens were embedded in OCT and used for DIF. After sectioning, samples were covered with OCT and archived at –20°C prior to being used for DNA extraction.

DNA Extraction

DNA was extracted and purified as previously described by Yehia et al. [8] with minor modifications. Five to ten cryosections of 10 μm were harvested from each OCT block in precooled tubes and lysed in 400 μl cell lysis solution (Qiagen). 30 μg of proteinase K was added to each sample. Following brief vortexing, the samples were incubated overnight under shaking at 56°C. After a 30-min incubation at 37°C with RNAse A (20 μg/tube), samples were spun at 13,000 *g* for 5 min to remove residual debris. The supernatant was collected and cooled on ice for 1 min, and 133 μl of protein precipitation solution (Qiagen) were added. After vortexing, samples were left on ice for at least 20 min and then centrifuged at 13,000 *g* for 5 min. The supernatant containing DNA was transferred to a new tube, and the DNA was precipitated by the addition of 400 μl of 100% isopropanol and 1 μl of glycogen solution (20 mg/ml; Qiagen). After mixing and centrifugation at 13,000 *g* for 5 min, the pellet of DNA was washed twice with 70% ethanol, dried and resuspended in 30 μl of 10 mM Tris buffer, pH 8.5.

PCR

PCR was performed with genomic DNA using 35 cycles for each primer set with Taq DNA polymerase (Life Technologies) in a final volume of 25 μl. Water instead of DNA template was used for negative controls. The β-globin gene (HBB) was amplified using the GH20 (5′-GAA GAG CCA AGG ACA GGT AC-3′) and PCO4 (5′-CAA CTT CAT CCA CGT TCA CC-3′) primer sets [9] with the following PCR conditions: 96°C/30 s, 55°C/30 s, 72°C/30 s. The type I collagen gene (COL1A1) was amplified using the ex48 (5′-CCA CCT CAA GAG AAG GCT CAC GA-3′) and re52 (5′-TGG GAT GGA GGG AGT TTA CA-3′) primer sets [10] with the following PCR conditions: 96°C/30 s, 55°C/30 s, 72°C/95 s.

Table 1. DNA was extracted from different specimens for each of the various transport media

Sample ID	Transport medium	Sex	Age, years	DNA, ng/µl	A_{260}	A_{280}	$A_{260/280}$	PCR HBB 268 bp	PCR COL1A1 1,360 bp
MM1	Michel's medium	M	63	17.92	0.358	0.199	1.8	positive	positive
MM2	Michel's medium	F	41	9.96	0.199	0.086	2.32	positive	positive
MM3	Michel's medium	F	79	3.35	0.067	0.026	2.57	positive	positive
MM4	Michel's medium	F	38	26.16	0.523	0.289	1.81	positive	positive
MM5	Michel's medium	M	91	6.92	0.138	0.071	1.95	positive	positive
MM6	Michel's medium	M	91	12.13	0.243	0.113	2.14	positive	positive
MM7	Michel's medium	M	63	23.06	0.461	0.263	1.75	positive	positive
MM8	Michel's medium	M	57	16.59	0.332	0.184	1.8	positive	positive
MM9	Michel's medium	F	75	12.39	0.248	0.148	1.68	positive	positive
MM10	Michel's medium	M	35	7.47	0.149	0.062	2.41	positive	positive
MM11	Michel's medium	F	89	6.41	0.128	0.071	1.81	positive	positive
MM12	Michel's medium	F	83	7.65	0.153	0.069	2.2	positive	positive
LN1	Liquid nitrogen	M	61	43.24	0.865	0.467	1.85	positive	positive
LN2	Liquid nitrogen	M	57	13.12	0.262	0.133	1.97	positive	positive
LN3	Liquid nitrogen	F	59	7.98	0.16	0.069	2.32	positive	positive
LN4	Liquid nitrogen	F	53	15.18	0.304	0.169	1.79	positive	positive
LN5	Liquid nitrogen	F	45	21.72	0.434	0.239	1.82	positive	positive
LN6	Liquid nitrogen	M	72	13.42	0.268	0.182	1.47	positive	positive
LN7	Liquid nitrogen	M	78	20.73	0.415	0.23	1.8	positive	positive
LN8	Liquid nitrogen	F	51	8.02	0.16	0.077	2.08	positive	positive
LN9	Liquid nitrogen	M	38	6.25	0.125	0.067	1.87	positive	positive
LN10	Liquid nitrogen	M	59	10.34	0.207	0.109	1.9	positive	positive
LN11	Liquid nitrogen	M	33	9.71	0.194	0.106	1.83	positive	positive
LN12	Liquid nitrogen	F	58	5.43	0.109	0.052	2.1	positive	negative
NaCL1	0.9% NaCl	F	66	6.33	0.127	0.056	2.26	positive	negative
NaCL2	0.9% NaCl	M	61	11.39	0.228	0.12	1.9	positive	positive
NaCL3	0.9% NaCl	F	66	28.72	0.574	0.305	1.88	positive	positive
NaCL4	0.9% NaCl	F	51	48.78	0.976	0.52	1.88	negative	positive
NaCL5	0.9% NaCl	M	53	11.23	0.225	0.116	1.94	positive	positive
NaCL6	0.9% NaCl	M	38	13.17	0.263	0.134	1.96	positive	positive

Absorbance was measured by spectrophotometer (Nanodrop ND1000), and nucleic acid sample purity was assessed by ratios of absorbance at 260 nm/280 nm ($A_{260/280}$). $A_{260/280}$ of 1.8 or greater is generally accepted as 'pure' for DNA. If the ratio is appreciably lower, it may indicate the presence of protein or other contaminants that absorb strongly at or near 280 nm.

Results

Extracted DNA was quantified by absorbance and evaluated for impurities by ratios of absorbance at 260 nm/280 nm ($A_{260/280}$) for residual protein contaminants. As shown in table 1, the DNA yield varied considerably among the different samples, probably because the cross-sectional tissue area and number of sections used for DNA extraction were different. An average of 14.6, 12.5 and 19.9 ng/µl of DNA was obtained for biopsies transported in liquid nitrogen, Michel's medium or normal chloride, respectively. The transport medium did not significantly influence sample purity as measured by $A_{260/280}$ ratios, with an average ratio above 1.8 for all of them indicating a minor contamination of DNA by proteins (table 1).

Amplification of small PCR products is sufficient for the diagnosis of skin infection such as syphilis, lyme borreliosis, leishmaniasis or tuberculosis as well as for lymphoma [11, 12]. Therefore, we assessed whether the quality of the extracted DNA was sufficient to allow PCR amplification of a 268-bp DNA fragment from the HBB gene and of 1,360 bp from the COL1A1

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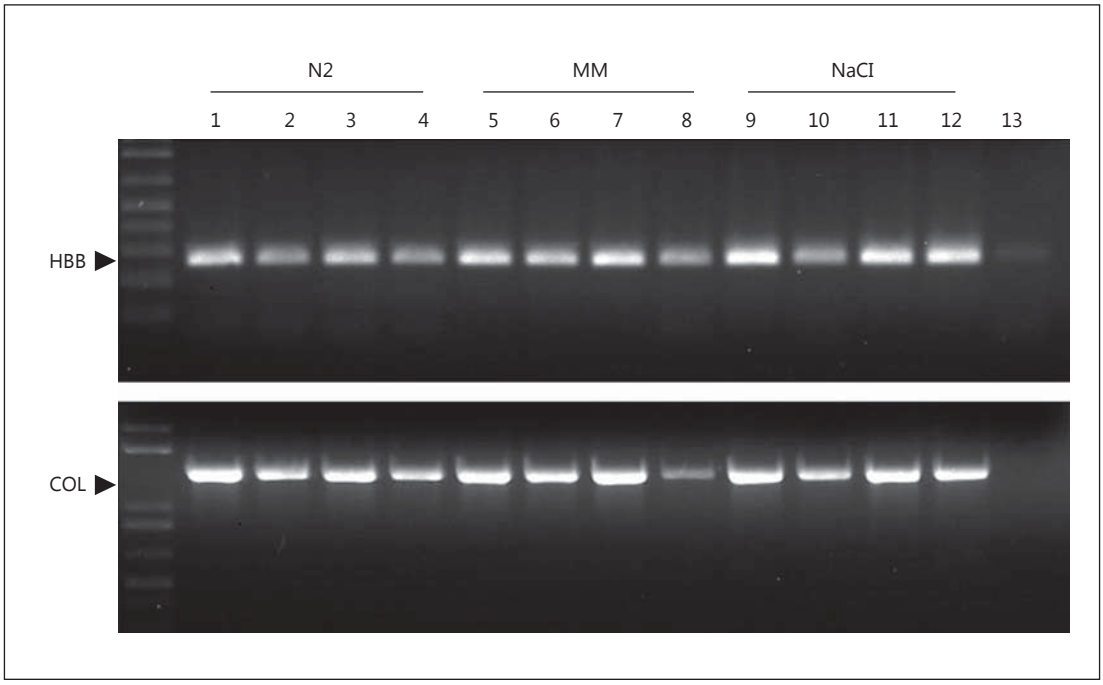

Fig. 1. Agarose gel electrophoresis of PCR amplicons after amplification of the HBB gene and COL1A1 gene (COL) from DNA prepared from skin biopsies transported in liquid nitrogen (N2), Michel's transport medium (MM) or normal saline (NaCl). 40 ng (lanes 1, 3, 5, 7, 9, 11) or 8 ng (lanes 2, 4, 6, 8, 10, 12) of total DNA was used as template in PCR. Lane 13 = Negative control.

gene. In a first series of PCR, 2 µl of purified DNA were used for PCR. As shown in table 1, most samples gave positive results for both genes, indicating that the transport medium did not significantly influence PCR efficacy. Next, PCR reactions were performed using a similar amount of template, 40 and 8 ng, from each of the fixation methods to emphasize any differences in sample quality. As shown in figure 1, all samples gave positive results with 40 and 8 ng of template.

Discussion

Michel's medium is a well-established transport medium used to maintain tissue-fixed immunoreactants prior to DIF, immunoelectron microscopy and epitope mapping for genodermatoses [13]. This transport medium probably preserves immuno-antigenicity by its ability to precipitate macromolecules while inhibiting proteolytic enzymes [2]. Here we report that no significant differences exist among liquid nitrogen, Michel's medium and normal chloride for short-term preservation of DNA for PCR analysis. We showed that as little as 8 ng of template DNA purified from sample in Michel's transport medium are sufficient for PCR amplification of amplicons larger than 1 kb. This contrasts with the results obtained with FFPE samples with formalin fixation causing fragmentation of nucleic acids that results in amplicons no larger than 500 bp [1, 14]. Furthermore, we found no obvious inhibitory effects of Michel's medium on PCR efficacy in the 12 tested samples. Thus, Michel's medium appears to be a reserve medium for performing PCR when no other samples are available.

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