Short Communication

Stability of JC Virus DNA in Cerebrospinal Fluid Specimens Preserved with Guanidine Lysis Buffer for Quantitative PCR Testing

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SUMMARY: Quantitative PCR testing for JC virus (JCV) DNA in the cerebrospinal fluid (CSF) is one of the diagnostic standards for progressive multifocal leukoencephalopathy (PML). The present study was conducted to examine its reliability using CSF specimens that had been preserved with guanidine lysis buffers in commercial nucleic acid extraction kits under different conditions. When CSFs were mixed with guanidine buffers, JCV DNA levels were not statistically reduced even after storage for 1 month at room temperature or for 3 months at -80°C , compared with the control samples. In addition, the JCV DNA level was not decreased in a mixture of CSF and guanidine thiocyanate buffer incubated for 3 days at 56°C . These data suggest that CSF specimens mixed with commercial guanidine buffers can be stored without refrigeration, more safely handled, and directly subjected to JCV DNA testing for PML.

The JC virus (JCV) is a small DNA virus belonging to the family *Polyomaviridae* and genus *Polyomavirus* (1–3). It is the causative agent of progressive multifocal leukoencephalopathy (PML), a fatal demyelinating disease of the central nervous system (1–3). PML is mainly diagnosed in patients with immunodeficiency, including those with HIV infection (1–3). The detection of JCV DNA in the cerebrospinal fluid (CSF) by PCR is a reliable and less-invasive diagnostic marker of PML, particularly when combined with typical magnetic resonance imaging patterns (1–3). The amount of JCV DNA in CSF has been reported to be associated with the severity of PML (4,5). Thus, JCV DNA testing in CSF using a sensitive and quantitative PCR technique has become one of the current diagnostic standards (6).

Real-time PCR testing for viral DNA in CSF is generally performed on DNA extracts prepared by either manual or automated methods using commercially available extraction kits, most of which contain guanidinium salts as one of their components (7). Guanidinium salts such as guanidine hydrochloride (Gdn-HCl) and guanidine thiocyanate (Gdn-SCN) are widely used for cell lysis and protein solubilization (7). These compounds inhibit nuclease activities (8,9) or inactivate infectious pathogens (10–14). If viral DNA in CSF is stable when mixed with a solution containing guanidine such as Gdn-HCl and Gdn-SCN, the specimen could be stored and transported without cooling and directly used for DNA extraction. Furthermore, CSF mixed with a guanidine solution can be considered to be a noninfectious material. The present study was undertaken to examine the stability of JCV DNA in CSF specimens preserved with guanidine lysis buffer.

The study was approved by the Ethical Committee for Biomedical Science in the National Institute of Infec-

tious Diseases, Tokyo, Japan (application no. 340). All the experiments were conducted in accordance with the ethical standards of the Declaration of Helsinki. All the CSF specimens were collected by lumbar puncture from patients suspected of having PML and were transferred from the respective hospitals to the Department of Virology 1, National Institute of Infectious Diseases, for real-time PCR testing for JCV DNA (15). Because all the specimens had been frozen without centrifugation at the hospitals, they were considered to contain leukocytes. The specimens were stored at -80°C until further analyses. Total DNAs were extracted from aliquots of the CSF specimens using the QIAamp DNA Blood Mini Kit (Qiagen, Valencia, CA, USA) and were subjected to real-time PCR testing for JCV DNA, as described previously (15–17).

To prepare a CSF pool for the experiments, JCV-negative CSF specimens from 71 patients were combined in a single tube (approximately 0.5 mL/sample). The median values of CSF cell counts and protein levels in the samples were 3.0 cells/ μL (range, 0–95.0 cells/ μL) and 48.0 mg/dL (range, 23.0–226.0 mg/dL), respectively. A JCV-positive CSF sample from an AIDS-related PML patient was diluted by more than 100-fold with JCV-negative CSF. In the JCV-positive patient, although the CSF cell count was normal (1 leukocyte/ μL), a relatively high level of the JCV genome (2.7×10^6 copies/mL CSF) was detected (15). Thus, it was thought that cell-free virions were dominant in this CSF pool. The copy number of JCV DNA in this CSF pool was adjusted to 2.0×10^4 copies/mL, which is similar to the median JCV load in CSFs of 69 PML patients in Japan (15). The CSF pools were aliquoted (200 or 140 μL) into 1.5-mL screw-cap plastic microcentrifuge tubes (Ina-Optica Co. Ltd., Osaka, Japan) and frozen at -80°C until use.

The column-based nucleic acid extraction kits and guanidine lysis buffers were all purchased from Qiagen. A 200- μL aliquot of the frozen CSF pool was thawed at 37°C in a block incubator (CTU-Mini; Taitec, Saitama, Japan), left untreated or mixed with an equal volume of

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Fig. 1. Schematic representation of the handling procedures for DNA extraction and the storage conditions of the mixtures of CSF and guanidine. Aliquots of the JCV-positive CSF pool were incubated with either Buffer AL or Buffer AVL. After incubation at different temperatures and for different durations, total DNA was extracted using a QIAamp DNA Blood Mini Kit or QIAamp Viral RNA Mini Kit as described in the text.

lysis buffer containing Gdn-HCl (Buffer AL), and stored with protection from exposure to light. Whether DNA recovery is decreased by visible light during storage in Buffer AL or Buffer AVL remains unclear. However, because the laboratory equipment was routinely irradiated with ultraviolet light to prevent DNA contamination before and after examination, we placed samples in lightproof boxes for long storage periods.

The handling procedures of the CSF guanidine solutions and storage conditions are summarized in Fig. 1. The frozen samples were thawed at 37°C and mixed thoroughly after storage. All the samples were incubated at 37°C for 10 min and then vortexed for 1 min. Subsequently, protease solution was added to the mixture, and total DNA was extracted using the QIAamp DNA Blood Mini Kit according to the manufacturer's protocol. Alternatively, 140-µL aliquots of the frozen CSF pool were thawed at 37°C and were left untreated or mixed with 560 µL of lysis buffer containing Gdn-SCN (Buffer AVL) and subsequently incubated under similar conditions. Total DNA was then extracted using the QIAamp Viral RNA Mini Kit according to the manufacturer's protocol, with the exception that carrier RNA was added to the lysate after incubation at 37°C for 10 min and that DNA was eluted in 60 µL of Buffer AE. Although Buffer AL and Buffer AVL were supplied in

the QIAamp DNA Blood Mini Kit and QIAamp Viral RNA Mini Kit, respectively, both the kits used the same extraction columns and wash buffers. Thus, after the lysates were passed through the columns, DNAs could be extracted using a common procedure. In the control experiments without additional incubation, the frozen aliquots of the CSF pool were thawed at 37°C just before use, and total DNAs were immediately extracted by standard procedures using the QIAamp DNA Blood Mini Kit and QIAamp Viral RNA Mini Kit. The extraction kits were routinely checked for their quality by adding plasmid DNA solution to the lysis buffer (control experiments). The contamination of control plasmid DNA into test samples can be detected using specific primers and probe (15).

The JCV DNA copy number in each sample was determined in duplicate using real-time PCR targeting the JCV large T gene, as described previously (15–17). The absence of DNA contamination was confirmed by means of negative controls. The absolute copy numbers of JCV DNA in each sample were determined. The experiments were repeated six times, and the amounts of JCV DNA in the extracts under the respective conditions were statistically compared using the nonparametric Steel test for multiple comparisons. All *P*-values less than 0.05 were considered to be statistically significant.

The absolute copy numbers of JCV DNA in the CSF pool, as determined by the standard procedures for the QIAamp DNA Blood Mini Kit and QIAamp Viral RNA Mini Kit, were 1.92×10^4 and 3.6×10^4 copies/mL, respectively. When CSFs were mixed with Buffer AL containing Gdn-HCl, JCV DNA levels were statistically reduced after incubation at 56°C for 3 days (Fig. 2A). However, there were no statistically significant differences between the control and Buffer AL-incubated samples stored under other conditions. In CSFs mixed with Buffer AVL containing Gdn-SCN, the amounts of JCV DNA were not statistically reduced after incubation under all conditions tested, although the viral DNA level was statistically lower when the specimens were incubated without lysis buffer at 27°C for 1 week and DNA was extracted by the standard procedure (Fig. 2B). No thermal deposition of guanidinium salt was observed in any lysate during incubation at 4°C for 1 month.

The results of the present study demonstrated that if CSF specimens are mixed with commercially available guanidine lysis buffers, they can be used for quantitative PCR testing for JCV DNA even after storage for 1 month at room temperature. In addition, it was also shown that lysates could be frozen to meet longer storage requirements. The target fragments of JCV DNA were demonstrated to be stable in the presence of Gdn-HCl or Gdn-SCN and other buffer components. However, JCV DNA levels were impaired in CSFs incubated with Buffer AL at 56°C for 3 days. This temperature is recommended for the proteinase K digestion step in the QIAamp DNA Blood Mini Kit protocol (56°C for 10 min) and is used to examine the stability of JCV DNA at a higher temperature. The present data suggest that JCV DNA in Buffer AL tends to be degraded at relatively high temperatures. It is important to examine the stability of purified viral DNA under more precise conditions in future studies. It was also found that JCV

Fig. 2. Availability of CSF specimens preserved with guanidine-containing lysis buffers for the quantitative PCR testing for JCV DNA detection. Aliquots of the JCV-positive CSF pool were incubated with (+) or without (-) either Buffer AL (A) or Buffer AVL (B) under the different conditions, and total DNA was extracted as described in the legend to Fig. 1. When CSFs were incubated without lysis buffer, the samples were mixed with lysis buffer after time periods and were subjected to DNA extraction. The absolute copy numbers of the JCV genome in each DNA extract were determined using a real-time PCR method. The experiments were repeated independently 6 times, and the results are shown in box-and-whisker plots. The thick horizontal line within each box is the median; the lower and upper boundaries are the 25th and 75th percentiles, respectively; vertical whiskers extend over the range; and no outliers were detected. Statistically significant differences ($P < 0.05$) are indicated by asterisks (comparison to the first box from the left).

DNA in CSF can be extracted using the QIAamp Viral RNA Kit designed for RNA extraction from liquid samples. The data obtained in the present study suggest that the QIAamp Viral RNA Kit containing Buffer AVL can be used to extract JCV DNA from liquid materials according to the manufacturer's instructions.

Compared with the results presented in Fig. 2A and 2B, it seems likely that the median copy numbers of JCV DNA in Buffer AVL have less variation under different conditions than those in Buffer AL. These results may be related to the higher initial amount of the obtained JCV DNA in Buffer AVL (3.6×10^4 copies/

mL) than that in Buffer AL (1.92×10^4 copies/mL), probably because of the addition of carrier RNA in the procedure. To examine the copy number of JCV DNA for PML diagnosis, it is important to obtain the amount of JCV DNA with less variation from samples containing the same amounts of JCV DNA under various conditions. Based on the above-mentioned findings, it seems likely that Buffer AVL is more applicable to the JCV DNA sample in CSF.

In addition, the current protocol using Buffer AVL is advantageous not only for sample storage as a part of routine JCV testing but also for the transportation of CSF specimens from outlying clinics to laboratories, without the need for a refrigerant. Considering the ability of Gdn-SCN to inactivate infectious agents, it is thought that the use of guanidine lysis buffer can be advantageous in decreasing the infectivity of pathogens. The results of the present study are thought to demonstrate that the use of a guanidine lysis buffer is a safe and convenient method for storing and handling CSF specimens. Furthermore, the preservation and storage of cell-free JCV suspension with guanidine solution may be useful for the *in vitro* high-throughput quantification of JCV DNA in a large number of samples.

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Conflict of interest None to declare.

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