

# Blood-Stained Letter Received by HIV/AIDS Reference Laboratory in 1993 – A Historical Case Report

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## ABSTRACT

*We describe an interesting historical case of a blood-stained letter received in 1993 by Slovenian HIV/AIDS Reference Laboratory. According to the statement of the sender, the letter was spotted with HIV-infected blood. The polymerase chain reaction (PCR) amplification with two gag and env primer sets excluded the presence of HIV proviral DNA in the spot punches obtained from the dried blood spots. Although the presence of HIV DNA was confirmed in 5 positive control blood spots using the same sets of primers, we still doubted in the accuracy of the HIV negative result. Fortunately, we obtained a serum sample of the author of the letter four months later from a psychiatric institution where he was hospitalized for paranoid schizophrenia. Since no anti HIV-1/2 antibodies were detected in his serum sample, our initial HIV negative findings based on PCR testing of blood spots were confirmed.*

**Key words:** human immunodeficiency virus, HIV, polymerase chain reaction, blood spot, blood stain

## Introduction

Considering serious psychological and social implications of being human immunodeficiency virus (HIV) positive, laboratory personnel performing HIV testing is under constant pressure, not only because of great responsibility due to possible incorrect diagnosis of HIV infection, but also because of relatively frequent unpleasant troubling by individuals without medical background. Subject to such troubling are especially employees of HIV/AIDS reference laboratories. Thus, we receive weekly telephone calls from concerned individuals tested recently for HIV infection in laboratories throughout Slovenia or in our HIV/AIDS Reference Laboratory demanding detailed explanations of their test results and our laboratory procedure used for exclusion of HIV infection. These questions are most frequently addressed by the individuals with indeterminate results of anti-HIV confirmatory tests. Duration of window period is another very frequent topic. For example, in January 2006, the same individual called our laboratory more than 10-times per day for a two-week period presenting himself under dif-

ferent names (even stating that he is medical doctor) asking about the details of our testing procedure and constantly doubting in his repeatedly negative anti-HIV status. Occasionally, we also receive letters of different content and length (the longest one contained ten pages), including threat letters from individuals claiming mistakes in our laboratory procedure and demanding legal rights for our supposed laboratory errors.

## Case Report

We received the most bizarre letter on March 22, 1993. This letter was sent in a white envelope covered with two small reddish spots that drew our special attention. We opened the envelope with precaution and found a 2-page letter in it, covered with more than 30 blood stains of different size with the total blood-covered area of approximately 120 square centimeters (Figure 1).

The author of the letter (M. S.) introduced himself with his full name and surname and wrote: »Since I am

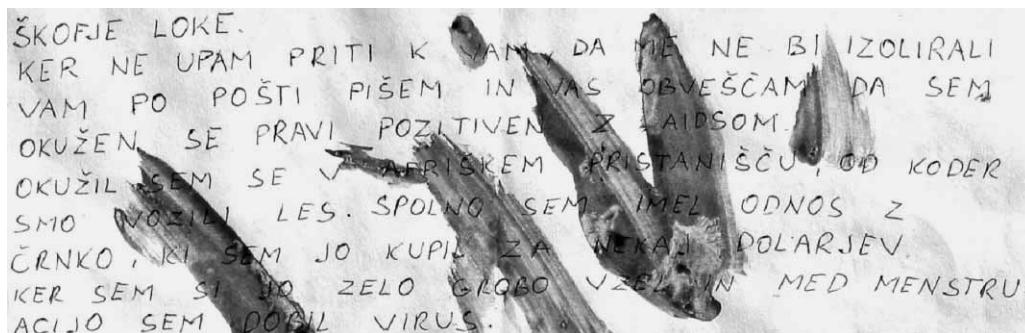


Fig. 1. A detail of blood-stained letter received by HIV/AIDS Reference Laboratory.

afraid of being detained and isolated if coming to your laboratory, I want to inform you by this letter that I am HIV positive and I have AIDS«. Later on, he described a rude sexual intercourse with a black female prostitute in African seaport where he was staying as a sailor, during which he was supposedly infected with HIV. On return to Italian seaport Bari, according to M. S., the captain of the ship ordered a HIV test for the entire crew. According to M. S., the testing showed that he is HIV positive. He claimed that, after this event, his life changed drastically. He wrote that he traveled to Kenya with a friend, where they had lived with a tribe of natives for 4 months and had sexual intercourse with several female members of the tribe. Presumably, they also witnessed a considerable number of people dying of AIDS and this greatly affected M. S. The following two months, M. S. and his friend continued their travel to Nigeria. As M. S. described in his letter, this occurred in 1987. After returning to Slovenia, he was experiencing alcohol and drug abuse and was involved in criminal activity. He claimed in the letter to have had occasional sexual intercourses and that he deliberately infected with HIV altogether 6 female and 6 male professional sexual workers. In conclusion of his letter, he threatened us not to search for him, because we would never find him, unless he would appear voluntarily. Interestingly, he signed the letter with his full name and surname including his full postal address.

Since M. S. was not identified in the Slovenian national register of anti-HIV positive individuals and no single M. S. blood sample could be retrieved from the serum/blood files at the Institute of Microbiology and Immunology, Faculty of Medicine, Ljubljana, Slovenia or any other Slovenian microbiology laboratory, we determined his HIV status by testing dried blood stains from his letter for the presence of HIV proviral DNA. For this purpose, one of the methods based on polymerase chain reaction (PCR) and originally developed for the detection of HIV in dried blood spots of newborns born to HIV positive mothers on filter paper or Guthrie cards was used<sup>1-3</sup>. For positive and negative controls, two drops of whole blood obtained from five Slovenian HIV infected individuals in different stages of HIV/AIDS and five anti-HIV negative Slovenian individuals, respectively, were spotted with a syringe onto the filter paper, air dried and stored at room temperature until use. Briefly, four blood

spot punches of each individual (measuring together app. 5 square mm) were suspended in the hemoglobin removal lysis buffer, and subsequently, DNA was extracted using the proteinase K/Tween-20/Nonidet P-40 method, as described in details previously<sup>1</sup>. For the detection of HIV DNA, all samples were tested using two different PCR protocols: nested PCR using the *gag* specific outer and internal primers SK380/390 and SK38/39, respectively<sup>1,4</sup>, and single-round PCR using the *env* specific primers SK68/69<sup>5</sup>, on a DNA Thermal Cycler 480 (Perkin-Elmer Cetus Instruments, Norwalk, USA), as described previously<sup>1</sup>. Additionally, the quality of DNA preparation and the absence of PCR-inhibitors were tested by the amplification of the 268 bp fragment of human beta-globin gene using the GH20 and PC04 primers, as described previously<sup>6,7</sup>. Amplification products were separated by electrophoresis using a 3% agarose gel prepared with 0.5xTBE buffer and were visualized by UV illumination following ethidium bromide staining. All precautions against specimen contamination and PCR carryover were rigorously taken at every step of testing<sup>8</sup>.

The internal control amplification with the human beta-globin primers GH20 and PC04 showed that the amplifiable DNA was recovered from all 10 control dried blood spots as well as from the blood stain from M. S. letter. The PCR amplification with primer sets targeting two different HIV genes showed the presence of HIV DNA in all five positive control specimens and absence of HIV DNA in all five negative control samples and in the blood stain from M. S. letter.

Although the PCR HIV DNA testing of the dried blood stains from M. S. letter didn't confirm the statement in his letter to be HIV positive and although we used the most reliable PCR primers available in that time due to the known huge genetic variability of HIV isolates, we were still in doubts of the reliability of our PCR method used for the exclusion of HIV infection. However, four months later (on July 29, 1993), we received a serum sample from Psychiatric Hospital Begunje ordering anti-HIV screening test for the patient with the same name and surname as M. S. The serum sample was tested for the presence of anti HIV-1 and anti HIV-2 antibodies using two different screening enzyme immunoassays (Wellcozyme HIV 1+2 Test, Murex Diagnostics, Dartford, Great Britain and Enzygnost Anti-HIV 1+2

Test, Behring AG, Marburg, Germany) and found to be clearly anti-HIV negative. Responsible psychiatrist from the Psychiatric Hospital Begunje was contacted and, after consultation, we undoubtedly confirmed that his patient and the author of the letter described above are one and the same person.

According to patient's history, M. S. was born in 1961 to working-class parents with low income. His father was treated as inpatient due to schizophrenia on several occasions, and in 1982, he was hospitalized under court order because of severe acts of violence. His parents were divorced in 1968, and afterwards, M. S. lived with his mother. When M. S. was two years old, he suffered from a brain concussion. Later on, he finished eight years of basic education and a vocational school; his motivation for schooling and his school performance were described as poor. He was employed as a worker, but has often changed his job himself or was dismissed. As a worker, he was disorderly and had poor relationship with his coworkers. In 1987, M. S. murdered an elderly woman. During forensic investigation, he was found to suffer from paranoid schizophrenia. In forensic expert's report, he was described as having systematized delusions, mostly of control and influence, and a formal thought disorder; probably he also had hallucinations (acoustic and cenesthetic). He also displayed pronounced negative symptoms as flat affect, loss of interests and social withdrawal. According to auto- and heteroanamnesic data, he had been to some extent psychotic for four or five years before the act of homicide; during this period, he also described homosexual behavior and alcohol abuse. He was continuously hospitalized from 1987 to 1990 in Psychiatric Hospital Begunje. In hospital records and in forensic expert's report there is no data whatsoever on

his alleged travel to Africa or being a sailor as described in his letter; even more, the quality of data excludes such a possibility completely (at least before 1990).

In July 1993, M. S. was once again hospitalized in Psychiatric Hospital Begunje for a psychotic episode. On admission, he was deluded, tense, and even aggressive. The intensity of his psychotic symptoms decreased markedly in three or four weeks. During this hospitalization, his serum sample was sent to our laboratory for anti-HIV testing. In hospital records, there is no data on patient's fears of having AIDS, but the most probable reason for anti-HIV testing (although not stated explicitly) was the request of the patient.

In conclusion, we described an interesting case of blood-stained letter that was received by HIV/AIDS Reference Laboratory in which we excluded HIV infection by the PCR testing of the blood spots. This represented a true challenge for our laboratory due to absence of commercial PCR-based HIV tests at that time. Fortunately, we obtained a serum sample of the author of the letter four months later. Since no anti HIV-1/2 antibodies were detected in his serum sample, our initial findings based on PCR testing of blood spots were confirmed.

### Addendum

To additionally verify the negative result of the PCR HIV DNA testing of the dried blood stains from M. S. letter performed in 1993, isolated DNA stored at  $-70^{\circ}\text{C}$  was tested again in May 2006 using modified procedure of Cobas Amplicor HIV Monitor 1.5 test (Roche Diagnostics, Mannheim, Germany) and an in-house real-time PCR, as described previously<sup>9,10</sup>. The results of both »modern« PCR HIV DNA tests were clearly negative.

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## **PISMO ZAMRLJANO KRVLJU POSLANO 1993. GODINE U REFERENTNI LABORATORIJ ZA HIV/AIDS – POVIJESNI PRIKAZ SLUČAJA**

### **S A Ž E T A K**

U radu je opisan zanimljiv povijesni prikaz slučaja pisma zamrljanog krvlju poslanog 1993. godine u Referentni laboratorij za HIV/AIDS Republike Slovenije. Iz sadržaja se moglo zaključiti da je pismo bilo zamrljano s krvlju zaraženom s HIV-om. Prisutnost provirusne HIV DNK u krvavim mrljama, isključena je uporabom dvije inačice lančane reakcije polimeraze (PCR) sa *gag* i *env* početnicama. Unatoč tome da je s istim početnicama prisutnost HIV DNK bila dokazana u kontrolnim mrljama krvi pet HIV-pozitivnih osoba, ostala je sumnja u ispravnost HIV negativnog rezultata. Na sreću, nakon četiri mjeseca je iz psihijatrijske ustanove u laboratorij poslan serumski uzorak autora pisma hospitaliziranog zbog paranoidne shizofrenije. Budući da je poslani serumski uzorak bio anti-HIV negativan, potvrđena je i pravilnost negativnih PCR rezultata dobivenih iz mrlja krvi.