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Detection of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in Pools of Sera Negative for Antibodies to HIV-1 and HIV-2

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A total of 234 pools were prepared from 10,692 consecutive serum samples negative for antibodies to human immunodeficiency virus type 1 (HIV-1) and HIV-2 collected at five virological laboratories (average pool size, 45 serum samples). Pools were screened for the presence of HIV-1 RNA by a modified commercial assay (Amplicor HIV-1 Monitor test) which included an additional polyethylene glycol (PEG) precipitation step prior to purification of viral RNA (PEG Amplicor assay). The sensitivity of this assay for HIV-1 RNA detection in individual serum samples within pools matches that of standard commercial assays for individual serum samples, i.e., 500 HIV-1 RNA copies per ml. Five pools were identified as positive, and each one contained one antibody-negative, HIV-1 RNA-positive serum sample, corresponding to an average of 1 infected sample per 2,138 serum samples. Retrospective analysis revealed that the five HIV-1 RNA-positive specimens originated from individuals who had symptomatic primary HIV-1 infection at the time of sample collection and who were also positive for p24 antigenemia. We next assessed the possibility of performing the prepurification step by high-speed centrifugation (50,000 × *g* for 80 min) of 1.5-ml pools containing 25 μl of 60 individual serum samples, of which only 1 contained HIV-1 RNA (centrifugation Amplicor assay). The sensitivity of this assay also matches the sensitivities of standard commercial assays for HIV-1 RNA detection in individual serum samples. The results demonstrate that both assays with pooled sera can be applied to the screening of large numbers of serum samples in a time- and cost-efficient manner.

Diagnosis of human immunodeficiency virus (HIV) infection is commonly based on the detection of antibodies to HIV, but seroconversion, i.e., the appearance of specific anti-HIV antibodies, usually occurs 3 to 8 weeks after the infectious contact and 5 to 10 days after the onset of symptoms associated with early infection (4, 9, 12, 19). The window period between infection and seropositivity can be shortened by testing plasma or sera for the presence of HIV p24 antigen (4, 6, 9) and/or HIV type 1 (HIV-1) RNA (4, 8, 13). The presence of HIV-1 RNA in plasma is a more sensitive marker than the presence of p24 antigen in plasma (4, 8), and several commercial kits are now available for the detection of HIV-1 RNA, including quantitative PCR (Amplicor), nucleic acid sequence-based amplification, and branched DNA signal amplification (Quantiplex) (21). However, although these techniques are routinely used for the determination of viremia in HIV-1-infected individuals, they cannot be used for systematic screening for HIV-1 infection due to their high costs and labor-intensive nature. Assays for HIV-1 RNA with pooled sera instead of individual sera, as performed previously for HIV-1 antibody testing (15, 25), would be less expensive and time-consuming but would carry the risk of reduced analytical sensitivity. We recently developed a boosted version of the Amplicor assay with a lower detection limit of 20 HIV-1 RNA copies per ml (23). The increase in sensitivity was achieved by introducing a high-speed centrifugation step prior to purification of viral RNA. In this assay, standard high-speed centrifuges restrict the input volume of samples to 1.5 ml.

For the present investigation, which was aimed at detecting antibody-negative HIV-1 RNA-positive samples among sera sent to microbiological laboratories, we developed two modified formats of the Amplicor assay for the analysis of pooled sera. The first was designed to allow low-speed centrifugation of large serum pools by using a polyethylene glycol (PEG) precipitation step prior to viral purification. The second was based on high-speed centrifugation of smaller input volumes.

MATERIALS AND METHODS

Collection of specimens. Between November 1996 and March 1997 a total of 10,692 individual serum samples were collected at five centers including four university hospital laboratories (Basel, Bern, Lausanne, and Geneva, Switzerland) and one private laboratory (Bio-Analytique Institute [BAI], Geneva). The commercial kits used for the detection of anti-HIV antibodies were the following: HIV1/2 AxSYM, (Abbott, Delkenheim, Germany) (Basel, Bern, BAI, and Geneva), VIDAS (BioMérieux, Marcy-l'Etoile, France) (Basel and Bern), GENSCREEN HIV1/2 (Sanofi Pasteur, Marnes la Coquette, France) (Basel, Lausanne, and Geneva), Cobas core anti-HIV1/HIV2 EIA DAGS (Roche, Basel, Switzerland) (Lausanne), and MUREX HIV1/2 ICE 1.0.2 (MUREX, Dartford, England) (BAI). All of the centers except BAI used two different screening tests for each serum sample; BAI used only one, either the MUREX assay or the Abbott assay. Pools were prepared daily by mixing a maximum of 70 individual HIV-1 antibody-negative serum samples (200 μl of each sample), stored at −75°C, and sent on dry ice once a week to the Geneva Laboratory of Virology. Lists of the samples included in the pools were recorded on the daily protocol used to perform antibody screening. Individual serum samples were stored at −20°C according to the standard procedure in use in each of the participating laboratories and were available for further analysis on request. Assays for HIV-1 p24 antigen in individual serum samples were performed retrospectively with a commercial kit (HIVAG-1 monoclonal; Abbott).

Four serum samples from HIV-1 seroconverters collected at the Geneva Laboratory were used to compare the Amplicor assay with the centrifugation Amplicor assay.

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TABLE 1. Detection of low levels of HIV-1 RNA in pools by PEG Amplicor assay

Amt of input HIV-1 RNA (copies/tube)[a]	Range of OD for HIV-1 RNA	DF[b]	Range of OD for IQS	DF	No. of pools positive for RNA/ total no. tested
100	1.31–>3.0	1	2.05–2.59	5	5/5
50	1.57–>3.0	1	2.65–>3.0	5	5/5
0	0.06–0.08	1	2.58–>3.0	5	0/5

[a] Quintuplicate pools each containing 59 HIV-1 RNA-negative serum samples (100 μl of each serum sample) provided by the Geneva Transfusion Center were mixed with 100 μl of a dilution of a serum sample with a known HIV-1 RNA copy number. During the same experiment a 1/20 dilution of an HIV-1-positive serum sample included in the pool was tested in triplicate by the HIV-1 Amplicor Monitor assay and was found to have 1,954 HIV-1 RNA copies/ml; the expected value was 2,000 copies/ml.

[b] DF, dilution factor (used for detection).

HIV-1 RNA assays. (i) PEG Amplicor assay. The PEG Amplicor assay is based on modifications of the Amplicor HIV-1 Monitor test (Roche) (17, 23) including (i) a PEG precipitation step prior to the extraction of viral RNA in order to accommodate larger input volumes and allow initial centrifugation at low speed (1,500 × *g*); (ii) an increase in the PCR amplification step from 30 to 40 cycles in order to achieve a higher sensitivity (qualitative assay); and (iii) a modification of reagent volumes in order to reach a final concentration of the internal quantitation standard (IQS) identical to that used in the standard Amplicor HIV-1 Monitor and to increase the concentration of RNA introduced in the PCR. Briefly, the pools of sera from the same participating center prepared daily were thawed, the tubes were centrifuged at 1,500 × *g* for 10 min to remove fibrin deposits, and half of the pooled volume (corresponding to 100 μl of each serum sample) was used for the detection of HIV-1 RNA. When the pools contained less than 20 serum samples they were mixed with other pools from the same center to yield an input volume of 3 to 7 ml. A 50% commercial PEG solution (Polyethylene glycol 6'000 Solution; Fluka Chemie AG, Buchs, Switzerland) and a 3 M NaCl solution (final concentration of PEG, 3%; final concentration of NaCl, 0.15 M) were added, and the tubes (15-ml Falcon tube; Becton Dickinson, Franklin Lakes, N.J.) were placed on ice for 30 min and spun at 1,500 × *g* for 30 min at 4°C. The resulting pellet, corresponding to about 100 μl, was mixed with 1,650 μl of Working Lysis Reagent (containing the IQS) and kept at room temperature for 10 min, and then 1,750 μl of isopropanol was added. The tubes were centrifuged at 1,500 × *g* for 15 min at room temperature, and the pellet was washed with 1 ml of 70% ethanol and then transferred to a 1.5-ml tube and spun at 12,500 × *g* for 5 min with a microcentrifuge. The ethanol was then removed completely, and the pellet was resuspended in 100 μl of Specimen Diluent. For the PCR, 50 μl of the processed specimen was mixed with 50 μl Master Mix and was amplified according to the manufacturer's instructions with a Perkin-Elmer 9600 thermal cycler (Roche, Branchburg, N.J.) with 36 cycles of 10 s at 95°C, 10 s at 60°C, and 10 s at 72°C instead of 26 cycles (40 total cycles instead of 30 total cycles). The detection of amplified sequences was performed according to the manufacturer's instructions, except that amplified samples were diluted only 1/1, 1/5, and 1/25 for the detection of HIV-1 RNA and 1/5 for the detection of the IQS. This allowed the inclusion of two samples per slot and thus a reduction in the cost. Pools were considered positive when the optical density for HIV-1 was >0.2 and when the OD for the IQS was >0.3. When HIV-1 RNA was detected, the pools of sera prepared daily were analyzed separately, and positive pools were split for further analysis (they were first split into pools of 10 serum samples, and then the individual serum samples were tested).

(ii) Centrifugation Amplicor assay. The centrifugation Amplicor assay is based on modifications of the Amplicor HIV-1 Monitor test and was performed with pools of 1.5 ml containing 59 HIV-negative serum samples and either 25 μl of an individual serum sample with a known HIV-1 RNA copy number or 25 μl of one serum sample from a seroconverting patient. Pools were centrifuged at 27,000 rpm (50,000 × *g*) for 80 min at 4°C with a Biofuge 28 RS centrifuge with a 3740 rotor with 12 positions (Heraeus AG, Osterode, Germany). The pellet was resuspended in 518 μl of Lysis Reagent and 82 μl of Working Lysis Reagent, and the mixture was then incubated for 10 min at room temperature. HIV-1 RNA was precipitated with 620 μl of isopropanol, spun at 12,500 × *g* for 15 min, washed with 1 ml of 70% ethanol, and spun again at 12,500 × *g* for 5 min. The ethanol was removed completely with a disposable transfer pipette, and the tubes were left open for 15 min under a closed box to remove traces of ethanol. The box was then treated with UV light overnight. The pellet was suspended in 55 μl of Specimen Diluent, and the processed specimen was treated as recommended by the manufacturer. Pools were considered positive when the OD for HIV-1 was >0.2 and when the OD for the IQS was >0.3.

(iii) Amplicor assay. The standard Amplicor HIV-1 Monitor test (Roche) was performed with individual serum samples according to the manufacturer's instructions.

RESULTS

PEG Amplicor assay. We first determined the optimal concentrations of PEG and NaCl required to achieve maximal recovery and ease of resuspension of the pellet and found that the addition of final concentrations of 3% PEG and 0.15 M NaCl was a good compromise (data not shown). A major goal was to achieve a degree of analytical sensitivity for individual samples within pools similar to that achieved by the Amplicor assay for individual samples tested separately. Table 1 reports the results for three series of pools of 6 ml each tested in quintuplicate and containing serum with known numbers of copies of HIV-1 RNA from the same infected individual. HIV-1 RNA was detected down to an input of 50 copies per pool, corresponding to a detection limit of 5 to 10 copies/ml. Because the pools contained only 0.1 ml of each serum sample, the detection limit for an individual serum sample within a pool was 500 copies/ml. A similar sensitivity was achieved when the same experiment was repeated twice with pools mixed with HIV-1 RNA containing sera from two other infected individuals (data not shown).

Screening of HIV-1/2 antibody-negative sera. A total of 234 pools containing 10,692 individual serum samples negative for antibodies to HIV-1 and HIV-2 (HIV-1/2 antibody-negative sera) were analyzed for the presence of HIV-1 RNA by the PEG Amplicor assay. Details concerning the number and size of the serum pools are reported in Table 2.

No inhibition of PCR amplification (OD for IQS, >1.0 at a dilution of 1/5) was detected for the 176 pools of sera collected at the four university hospital laboratories. Nine of 58 pools received from the private laboratory (BAI) had evidence of inhibition, as indicated by ODs for the IQS ranging from 0.108 to 0.253 at a dilution of 1/5. Further investigations revealed a protocol violation due to the inclusion of heparinized samples in the nine pools (heparin is known to inhibit PCR reactions [11]). These pools were among the first 20 collected at this laboratory. No inhibitory activity was detected in the next 38 pools provided by the same private laboratory. These pools contained only sera prepared according to the protocol.

Five pools were positive for HIV-1 RNA, all with an OD for HIV-1 of >1.0 at a dilution of 1/5 (Table 3). Initially, two additional pools had been positive for HIV-1 RNA, but further analysis revealed that they contained each an HIV-1/2 antibody-positive sample which had been included in the pool by mistake. The five pools each contained a single serum sample (from subjects 1 to 5) which was HIV-1 RNA positive and HIV-1/2 antibody negative, as confirmed by retesting of the

TABLE 2. Analysis of HIV-1 antibody-negative sera by PEG Amplicor assay

Center	No. of serum samples[a]	No. of pools[b]	Pool size[c]	No. of HIV-1 RNA-positive pools[d]
Basel	1,312	32	22–57 (41)	1
Bern	1,786	38	33–62 (47)	0
Lausanne	1,050	25	29–77 (42)	0
BAI	2,494[e]	58[e]	22–73 (43)	0
Geneva	4,050	81	18–67 (50)	4
Total	10,692	234	18–77 (45)	5

[a] Number of individual HIV-1 antibody-negative serum samples.

[b] Number of pools tested for HIV-1 RNA by the PEG Amplicor assay.

[c] Values are size range (average size) of pools, in number of samples.

[d] Number of HIV-1 RNA-positive pools detected.

[e] A total of 504 individual serum samples (nine pools) could not be evaluated due to inhibition of PCR amplification.

TABLE 3. Size and OD of the HIV-1-positive pools and clinical parameters for HIV-1-positive subjects[a]

Pool no. (no. of samples)	OD for HIV-1	Subject no.	Amt of HIV-1 RNA (copies/ml)[b]	p24 antigen concn (ng/liter)[c]
1.03 (52)	2.0	1	35,416,220	>200
2.12 (61)	>3.0	2	1,620,000	130
3.04 (48)	>3.0	3	1,029,167	87
4.04 (59)	>3.0	4	2,992,000	270
5.12 (18)	>3.0	5	8,006,675	7,700

[a] The dilution factor was 5 for all pools. The Western blot result was negative for all subjects.
[b] Viremia was determined in individual serum samples by the Amplicor assay (Roche).
[c] Detection of p24 antigen in individual serum samples was performed with the HIVAG-1 monoclonal kit (Abbott).

sera for the presence of HIV-1/2 antibody by three screening tests (Abbott HIV1/2 AxSYM, VIDAS BioMérieux, GENSCREEN HIV1/2). Western blot analysis was also negative for all five serum samples. The five serum samples were tested individually for HIV-1 RNA (Amplicor assay) and for the presence of p24 antigen and were positive by both assays (Table 3).

The five HIV-1 RNA-positive, HIV-1/2 antibody-negative serum samples were from individuals suffering from primary HIV-1 infection, as established on the basis of clinical symptoms (data not shown) and laboratory parameters (Table 3). Each individual presented with seroconversion, i.e., the appearance of HIV-1-specific antibodies in plasma, during the follow-up period. Subjects 1 to 3 had been infected as a result of heterosexual contact, subject 4 had been infected as a result of homosexual contact, and subject 5 had been infected as a result of intravenous drug abuse.

Centrifugation Amplicor assay. Since the PEG Amplicor assay is relatively complex, we developed a second, simpler assay for the detection of HIV-1 RNA in pooled sera. This assay is based on a previously described boosted Amplicor assay characterized by high-speed centrifugation prior to purification of viral RNA (23). The centrifugation Amplicor assay allows centrifugation of pools containing as many as 60 individual serum samples (25 μl of each sample) in 1.5-ml tubes, accommodated by standard high-speed centrifuges (e.g., Heraeus Biofuge 28 RS). The analytical sensitivity of the centrifugation assay is reported in Table 4. Four pools of 1.5 ml each containing 60 individual serum samples, 1 of which was from an HIV-1-infected individual and which had a known HIV-1 RNA copy number, were tested in quintuplicate. The results indicate that HIV-1 RNA was detected in all five pools containing approximately 12.5 copies and in four of five pools containing approximately 6.25 copies. No inhibition of PCR amplification was observed. Thus, the theoretical lower detection limit for individual sera within pools (input volume, 25 μl per serum sample) can be estimated to be 12.5 copies × 40 = 500 copies/ml, where 40 is a correction factor taking in account the 25 μl of volume of each serum sample introduced into the pool. Similar results were obtained when the same experiment was repeated twice with pools mixed with HIV-1 RNA containing either 10 or 5 copies provided by sera from two other HIV-1-infected individuals. HIV-1 RNA was detected in all pools containing 10 copies and in 59% of pools containing 5 copies (data not shown).

We compared the ability of the Amplicor assay to detect HIV-1 RNA in individual serum samples with that of the centrifugation Amplicor assay to detect HIV-1 RNA in pools of 60 serum samples. Four serum samples from seroconverting individuals, with HIV-1 RNA levels ranging between 133,400 and 1,101,900 copies/ml, were tested in duplicate: individually (200 μl) and mixed in a pool. Each pool contained 59 HIV-1 RNA-negative serum samples (1.475 ml) and 1 serum sample (25 μl) from a seroconverting subject. The ODs for HIV-1 RNA and IQS were similar in both the Amplicor and the centrifugation Amplicor assays (data not shown), indicating that the sensitivity of the centrifugation Amplicor assay matches the sensitivity of the commercial assay for specimens diluted 60 times.

DISCUSSION

The window period between HIV infection and its detection by laboratory methods poses a challenge for the diagnosis of HIV-1 infection (4, 6, 26, 27). The infectious window period defines the time during which an individual is both infected and infectious prior to seroconversion. While the sensitivities of HIV antibody detection assays have significantly improved since the tests were first licensed in 1985 (2, 4, 5, 10, 28), the infectious window period is still longer for the assays based on the detection of anti-HIV antibodies than for the assays based on the detection of viral nucleic acids. Indeed, the assays based on the detection of HIV-1 RNA by PCR gave positive results 7 to 11 days before the assays based on the detection of anti-HIV antibodies (4). However, PCR for HIV-1 RNA is too expensive and labor-intensive for routine diagnostic screening of single serum specimens. A reduction in cost might be achieved by using an assay with pooled samples, which reduces the amounts of both laboratory materials and the supplies required. The Amplicor HIV-1 Monitor kit for the detection of HIV-1 RNA costs about US$600. The kit allows 24 assays to be performed; i.e., each assay costs the laboratory about US$25. The patient pays about US$50 for an assay for HIV-1 RNA performed with a single serum sample (US$25 for the Amplicor HIV-1 Monitor kit and US$25 for additional costs). Considering a mean pool size of 45 serum samples, the assays with pooled sera described here reduce the cost of screening of a single serum specimen for HIV-1 RNA by a factor of 45, since only 1 assay (instead of 45 assays) needs to be performed. Moreover, the assays with pooled sera can be performed in a time-efficient manner. In 1 working day, using the PEG Amplicor assay, a trained technician can test 15 to 20 pools containing approximately 1,000 individual serum samples, i.e., 20 times more serum samples than can be tested individually.

This study demonstrates that PCR for HIV-1 RNA can be performed with large pools of sera for diagnostic purposes and

TABLE 4. Sensitivity of the centrifugation Amplicor assay

Amt of input HIV-1 RNA (no. of copies/tube)[a]	Mean (range) OD for HIV-1 RNA	DF[b]	Mean (range) OD for IQS	DF	No. of pools positive for RNA/total no. tested
50.0	0.49 (0.33–0.62)	5	0.80 (0.60–0.93)	5	5/5
25.0	0.27 (0.19–0.40)[c]	5	0.66 (0.53–0.82)	5	5/5
12.5	0.89 (0.42–1.81)	1	0.86 (0.76–1.00)	5	5/5
6.25	0.41 (0.16–0.64)[d]	1	0.81 (0.77–0.87)	5	4/5
0.00	0.07 (0.06–0.07)	1	0.80 (0.75–0.87)	5	0/5

[a] Pools of 1.5 ml containing 59 serum samples that were antibody and HIV-1 RNA negative were mixed with 25 μl of dilutions of serum from an HIV-1-infected individual with known numbers of HIV-1 RNA copies per milliliter and were tested in quintuplicate. During the same experiment a 1/20 dilution of an HIV-1-positive serum sample included in the pool was tested by the HIV-1 Amplicor Monitor test in triplicate, and 2,008 HIV-1 RNA copies/ml were found; the expected value was 2,000 RNA copies/ml.
[b] DF, dilution factor.
[c] For the only pool for which the OD was <0.2 (OD, 0.19) at a dilution of 1/5, the OD was 0.72 at a dilution of 1/1.
[d] The OD was <0.2 (OD, 0.16) for only one sample, i.e., was negative according to the manufacturer's instructions.

that the sensitivities of the PEG and centrifugation Amplicor assays for the detection of HIV-1 RNA in individual serum samples within pools are equivalent to the sensitivity of the Amplicor assay for the detection of HIV-1 RNA in single serum samples, i.e., 500 copies/ml. Both assays with pooled sera have a detection limit of between 5 and 10 copies/ml for total HIV-1 RNA in pools (23) (Tables 1 and 4) and thus are 5 to 10 times more sensitive than the recently modified, ultrasensitive Amplicor assays, which have detection limits of 50 copies/ml (18, 23). Of note, a very high analytical sensitivity is important for the detection of suppressed viremia in patients treated with potent antiretroviral drugs but not for the identification of HIV-1 RNA in individuals with primary HIV infection prior to seroconversion. These patients have elevated HIV-1 RNA levels, ranging between 10^3 and 10^8 copies/ml (3, 20).

The assays with pooled sera represent an alternative to additional diagnostic procedures selected to complement diagnoses based on a single antibody test. Thus, in countries such as France and Switzerland, it is recommended that microbiology laboratories screen individual serum samples by two commercial antibody-based assays. However, the assays with pooled sera for the detection of HIV-1 RNA are likely to reduce the window period during which infection can be detected to a greater extent than additional assays for p24 antigen and/or HIV antibodies. One of the temporary limitations of the present pooled serum assays based on the Amplicor assay is their inability to detect some of the non-B HIV-1 subtypes (1), but new PCR primers with a wider range of detection for non-B HIV-1 subtypes are being released by the Amplicor assay manufacturer (16).

According to the locally available equipment and local needs, laboratories may give preference to one of the pooled serum assays described here. The centrifugation Amplicor assay requires a high-speed centrifugation and the pipetting of small volumes (25 μl), and the pool size is limited to 60 serum samples. The PEG Amplicor assay may accommodate larger pools containing up to 80 serum samples, allows for easier pipetting due to the use of larger volumes (100 μl), and does not require additional equipment for centrifugation. Its main drawback is the necessity to transfer the pellet from a large 15-ml tube to a 1.5-ml tube.

Early recognition of primary HIV-1 infection has important implications for treatment, prognosis, prevention of disease dissemination in the community, and the safety of blood and tissue donations (4, 6, 7, 22, 24, 26, 27). The pooled serum assays described here with 10,692 consecutive HIV-1/2 antibody-negative serum samples sent to microbiology laboratories for HIV-1 antibody testing led to the identification of five individuals suffering from primary HIV-1 infection. This corresponds to an average of 1 infected serum sample per 2,138 serum samples. There may be two explanations for this relatively high incidence: first, the higher incidence of HIV-1-infected individuals in a population of people screened for HIV-1 infection compared to the incidence in the general population and, second, the relatively high prevalence of HIV-1 infection in Switzerland compared to that in other Western countries (14). All five individuals and their sexual partners likely involved in the transmission of the infection are receiving potent antiviral combination therapy. They have been informed of their infection status and how to protect their sexual partners. The early identification of subjects with primary HIV infection might contribute to a reduction in the spread of HIV.

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REFERENCES

1. **Alaeus, A., K. Lidman, A. Sönnerborg, and J. Albert.** 1997. Subtype-specific problems with quantification of plasma HIV-1 RNA. AIDS **11:**859–865.
2. **Ayres, L., F. Avillez, A. Garcia-Benito, F. Deinhardt, L. Gurtler, F. Denis, G. Leonard, S. Ranger, P. Grob, H. Joller-Jemelka, G. Hess, S. Seidl, H. Flacke, F. Simon, F. Brun-Vézinet, D. Sondag, A. André, H. Hampl, R. Schoen, S. Stramer, and H. Troonen.** 1990. Multicenter evaluation of a new recombinant enzyme immunoassay for the combined detection of antibody to HIV-1 and HIV-2. AIDS **4:**131–138.
3. **Baumberger, C., S. Kinloch-de-Loes, S. Yerly, B. Hirschel, and L. Perrin.** 1993. High levels of circulating RNA in patients with symptomatic HIV-1 infection. AIDS **7**(Suppl. 2)**:**S59–S64.
4. **Busch, M. P., L. L. Lee, G. A. Satten, D. R. Henrard, H. Farzadegan, K. E. Nelson, S. Read, R. Y. Dodd, and L. R. Petersen.** 1995. Time course of detection of viral and serologic markers preceding human immunodeficiency virus type 1 seroconversion: implications for screening of blood and tissue donors. Transfusion **35:**91–97.
5. **Busch, M. P., and A. M. Couroucé.** 1997. Relative sensitivity of United States and European assays for screening blood donors for antibodies to human immunodeficiency virus. Transfusion **37:**352–353.
6. **Couroucé, A. M., F. Barin, M. Maniez, C. Janot, L. Noel, and M. H. Elghouzzi.** 1992. Effectiveness of assays for antibodies to HIV and p24 antigen to detect very recent HIV infections in blood donors. Retrovirus Study Group of the French Society of Blood Transfusion. AIDS **6:**1548–1550. (Letter.)
7. **Coutlee, F., G. Delage, F. Lamothe, S. Cassol, and F. Decary.** 1992. Transmission of HIV-1 from seronegative but PCR-positive blood donors. Lancet **340:**59. (Letter.)
8. **De Saussure, P., S. Yerly, E. Tullen, and L. H. Perrin.** 1993. Human immunodeficiency virus type 1 nucleic acids detected before p24 antigenemia in a blood donor. Transfusion **33:**164–167.
9. **Galetto-Lacour, A., S. Kinloch-de-Loës, B. Hirschel, and L. Perrin.** 1995. Primo-infection VIH: un diagnostic souvent évoqué mais posé tardivement. Schweiz. Med. Wochenschr. **125:**341–346.
10. **Gallarda, J. L., D. R. Henrard, D. Liu, S. Harrington, S. L. Stramer, J. E. Valinsky, and P. Wu.** 1992. Early detection of antibody to human immunodeficiency virus type 1 by using an antigen conjugate immunoassay correlates with the presence of immunoglobulin M antibody. J. Clin. Microbiol. **30:** 2379–2384.
11. **Holodniy, M., S. Kim, D. Katzenstein, M. Konrad, E. Groves, and T. C. Merigan.** 1991. Inhibition of human immunodeficiency virus gene amplification by heparin. J. Clin. Microbiol. **29:**676–679.
12. **Horsburgh, C. R., C. Y. Ou, J. Jason, S. D. Holmberg, I. M. Longini, C. Schable, K. H. Mayer, A. R. Lifson, G. Schochetman, and J. W. Ward.** 1989. Duration of human immunodeficiency virus infection before detection of antibody. Lancet **ii:**637–640.
13. **Krivine, A., A. Yakudima, M. Le May, V. Pena-Cruz, A. S. Huang, and K. McIntosh.** 1990. A comparative study of virus isolation, polymerase chain reaction, and antigen detection in children of mothers infected with human immunodeficiency virus. J. Pediatr. **116:**372–376.
14. **Lederberger, B., J. von Overbeck, M. Egger, and R. Lüthy.** 1994. The Swiss HIV cohort study: rationale, organization and selected baseline characteristics. Soz. Präventivmed. **39:**387–394.
15. **McMahon, E. J., C. Fang, L. Layug, and S. G. Sandler.** 1995. Pooling blood donor samples to reduce the cost of HIV-1 antibody testing. Vox Sang. **68:** 215–219.
16. **Michael, N., M. Robb, D. Birx, J. R. Mascola, J. Wang, K. Dreyer, K. McDonough, C. Christopherson, S. D. Lu, S. Kwok, and S. Herman.** 1997. Performance of the Amplicor HIV-1 Monitor test and a modified HIV-1 Monitor test on HIV-1 subtypes A to F, abstr. 279. *In* Abstracts of the 4th Conference on Retroviruses and Opportunistic Infections.
17. **Mulder, J., N. McKinney, C. Christopherson, J. Sninsky, L. Greenfield, and S. Kwok.** 1994. Rapid and simple PCR assay for quantitation of human immunodeficiency virus type 1 RNA in plasma: application to acute retroviral infection. J. Clin. Microbiol. **32:**292–300.
18. **Mulder, J., R. Resnick, B. Saget, S. Scheibel, S. Herman, H. Payne, R. Harrigan, and S. Kwok.** 1997. A rapid and simple method for extracting human immunodeficiency virus type 1 RNA from plasma: enhanced sensitivity. J. Clin. Microbiol. **35:**1278–1280.
19. **Petersen, L. R., G. A. Satten, R. Dodd, M. Busch, S. Kleinman, A. Grindon, and B. Lenes.** 1994. Duration of time from onset of human immunodeficiency virus type 1 infectiousness to development of detectable antibody. The HIV Seroconversion Study Group. Transfusion **34:**283–289.
20. **Piatak, M., M. S. Saag, L. C. Yang, S. J. Clark, J. C. Kappes, K. C. Luk, B. H. Hahn, G. M. Shaw, and J. D. Lifson.** 1993. High levels of HIV-1 in plasma during all stages of infection determined by comparative PCR. Science **259:** 1749–1754.
21. **Revets, H., D. Marissens, S. de Wit, P. Lacor, N. Clumeck, S. Lauwers, and G. Zissis.** 1996. Comparative evaluation of NASBA HIV-1 RNA QT, AMPLICOR-HIV Monitor, and QUANTIPLEX HIV RNA assay, three methods for quantification of human immunodeficiency virus type 1 RNA in plasma. J. Clin. Microbiol. **34:**1058–1064.

22. **Schacker, T., A. C. Collier, J. Hughes, T. Shea, and L. Corey.** 1996. Clinical and epidemiologic features of primary HIV infection. Ann. Intern. Med. **125:** 257–264.
23. **Schockmel, G. A., S. Yerly, and L. Perrin.** 1997. Detection of low HIV-1 RNA levels in plasma. J. Acquired Immune. Defic. Syndr. Hum. Retroviral. **14:**179–183.
24. **Schreiber, G. B., M. P. Busch, S. H. Kleinman, and J. J. Korelitz.** 1996. The risk of transfusion-transmitted viral infections. The Retrovirus Epidemiology Donor Study. N. Engl. J. Med. **334:**1685–1690.
25. **Sherlock, C. H., S. A. Strathdee, T. Le, D. Sutherland, M. V. O'Shaughnessy, and M. T. Schechter.** 1995. Use of pooling and outpatient laboratory specimens in an anonymous seroprevalence survey of HIV infection in British Columbia, Canada. AIDS **9:**945–950.
26. **Simonds, R. J., S. D. Holmberg, R. L. Hurwitz, T. R. Coleman, S. Bottenfield, L. J. Conley, S. H. Kohlenberg, K. G. Castro, B. A. Dahan, C. A. Schable, M. A. Rayfield, and M. F. Rogers.** 1992. Transmission of human immunodeficiency virus type 1 from a seronegative organ and tissue donor. N. Engl. J. Med. **326:**726–732.
27. **Ward, J. W., S. D. Holmberg, J. R. Allen, D. L. Cohen, S. E. Critchley, S. H. Kleinman, B. A. Lenes, O. Ravenholt, J. R. Davis, M. G. Quinn, and H. W. Jaffe.** 1988. Transmission of human immunodeficiency virus (HIV) by blood transfusions screened as negative for HIV antibody. N. Engl. J. Med. **318:** 473–478.
28. **Zaaijer, H. L., P. van Exel-Oehlers, T. Kraaijeveld, E. Altena, and P. N. Lelie.** 1992. Early detection of antibodies to HIV-1 by third-generation assays. Lancet **340:**770–772.