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Standardisation of primers and an algorithm for HIV-1 diagnostic PCR evaluated in patients harbouring strains of diverse geographical origin

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Abstract

Eight Belgian AIDS Reference Laboratories established a multicentre quality control to evaluate the performance of their diagnostic human immunodeficiency virus type 1 (HIV-1) DNA polymerase chain reaction (PCR). A set of Belgian and African HIV-1 seropositive and seronegative patient samples, collected in Belgium, and the British Medical Research Council (MRC)

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HIV-1 PCR reference reagent kit, containing plasmid HIV-1 DNA at several dilutions in human carrier DNA with appropriate negative controls, were tested by the laboratories. No false positive results were reported. All laboratories were able to detect one to two copies of HIV-1 DNA. Among the 17 Belgian and African HIV-1 seropositives, some laboratories reported up to four indeterminate results, mainly due to failure of the SK38-39, SK68-69 (Ou et al. (1988) Science 239, 295–297) and/or gag881-882 (Simmonds et al. (1990) J. Virol. 64, 864–872) primers and a poorly performing algorithm. Only the H1POL4235-4538 nested *pol* primer set, developed by one of the laboratories, correctly identified all the tested HIV-1 positive and negative samples. Consequently, the laboratories decided to evaluate these *pol* primers as a reference primer set and to standardise the testing algorithm. All laboratories achieved a sensitivity and specificity of 100% on testing 10 additional Belgian and African patient samples, when adapting a standardised algorithm based on three HIV-1 primer sets, one of which is the H1POL4235-4538 primer set.

Keywords: HIV-1 DNA PCR; Multicentre quality control; African patients; Diagnosis of HIV-1

Introduction

Since the development of the highly sensitive polymerase chain reaction (PCR) for the amplification of a target DNA fragment, PCR has been used increasingly for the diagnosis of viral diseases, in particular for the detection of human immunodeficiency virus (HIV), the causative agent of AIDS (for a review see Clewley, 1989; Coutlée et al., 1991). PCR is especially useful as an additional technique when serological results are repeatedly doubtful (Genesca et al., 1989) or in situations where serology is not interpretable, i.e., for the diagnosis of HIV infection in neonates of infected mothers (Rogers et al., 1989; Garbarg-Chenon et al., 1993). PCR may also be used to detect proviral DNA and circulating viral RNA in the early stage of infection before antibodies become detectable (Loche and Mach, 1988; Imagawa et al., 1989). Additionally, PCR offers several advantages over virus culture: it requires shorter hands-on time and provides faster results, it uses a small sample volume, and with the appropriate primer pairs it is more sensitive than culture in asymptomatic individuals (Kellogg and Kwok, 1990).

The actual output from the PCR result to the clinician is highly dependent on the primers used (Lynch et al., 1992; Fransen et al., 1994), the type of HIV strain(s) (Grankvist et al., 1991), the testing algorithm (Wages et al., 1991) and the technical environment and expertise of the lab performing the PCR (Bootman and Kitchin, 1992). Therefore, throughout the reference laboratories, there is a need for standardising the diagnostic HIV-PCR protocol (Sheppard et al., 1991; Defer et al., 1992; Kitchin and Bootman, 1993).

In Belgium, eight AIDS reference laboratories were established. Their task is to confirm positive serology, to collect epidemiological data, to guide the follow up of patients, to assure the quality of diagnostic reagents and recently also to provide a diagnostic PCR for HIV. The multicentre study reported here was conducted to evaluate the methods, the primers and the algorithm used by the different laboratories, and to standardise the different steps in the diagnostic HIV-PCR protocol.

Materials and methods

2.1. Participating laboratories

The participating Belgian AIDS Reference Laboratories are: Katholieke Universiteit Leuven, Rega Instituut en Universitaire Ziekenhuizen, Laboratorium voor de Virusziekten; Instituut voor Tropische Geneeskunde, Laboratorium voor Microbiologie; Université Libre de Bruxelles, Hôpital Universitaire Erasme, Laboratoire de Virologie; Vrije Universiteit Brussel, Universitair Ziekenhuis St. Pieter, Laboratorium voor Virologie; Institut Pasteur du Brabant, Laboratoire de Virologie; Université de Liège, Centre Hospitalier Universitaire, Centre de Transfusion Sanguine; Université Catholique de Louvain, Unité de Microbiologie; Universitair Ziekenhuis Gent, Laboratorium voor Klinische Chemie, Microbiologie en Immunologie.

2.2. Samples used for the quality controls

The samples of quality control (QC) Nos. 2, 3 and 4 were collected in Belgian hospitals, and each prepared by a different participating laboratory. The cells were separated from the plasma by centrifugation and peripheral blood mononuclear cells (PBMCs) were prepared on a Ficoll gradient.

Quality control No. 1: samples A_1 to J_1: HIV-1 PCR reference reagent kit from the British Medical Research Council (MRC) (Bootman and Kitchin, 1992).

Quality control No. 2: samples A_2 to H_2 were frozen (dry ice) PBMC pellets ($2 \cdot 10^6$ cells/sample) from Belgian individuals. A_2, C_2: HIV-1 seropositive not confirmed by culture; B_2, D_2: HIV-1 seropositive and culture positive; E_2, F_2, G_2, H_2: healthy seronegatives.

Quality control No. 3: samples A_3 to G_3 were frozen (dry ice) PBMC pellets ($2 \cdot 10^6$ cells/sample); A_3: HIV-1 culture positive from Rwanda; D_3: from Zaire; E_3: from Togo; G_3: from Kenya; B_3, C_3, F_3: seronegatives from Belgium; H_3: cDNA sample from plasma of a seropositive from Kenya; K_3, J_3, I_3: cDNA samples from 10-fold serial dilutions of a Hut 78-$HTLV_{IIIB}$ culture supernatant; L_3: cDNA from plasma of a seronegative blood donor.

Quality control No. 4: samples A_4 to J_4 were frozen (dry ice) PBMC pellets ($1 \cdot 10^6$ cells/sample); A_4, B_4, E_4, F_4, G_4, H_4: from Belgian seropositives; C_4: from a seropositive from Zimbabwe visiting Belgium; D_4, I_4, J_4: from healthy Belgian seronegatives.

2.3. Testing procedure

Sample preparation

The cells were digested with proteinase K in PCR buffer or PBS containing detergent (Tween-80, Nonidet P-40, SDS or Sarcosyl), $MgCl_2$ and for some laboratories also gelatine or $(NH_4)_2SO_4$. The lysate was cleared by centrifugation and used directly or stored at $-20°C$. Four of the eight laboratories extracted the DNA with phenol/chloroform.

Primers and probes

All laboratories amplified a specific one-copy human gene to check the quality of the DNA: PC03-PC04, PC03-KM38 or PC04-GH20 amplifying globin (Saiki et al., 1988); MG21 5′-CGTCATCCAGCAGAGAATGG-3′ (sense nucleotides 149–168 of Hu β_2-microglobulin EMBL accession number (AC) M17987) and MG22 5′-AGTCACATG-GTTCACACGGC-3′ (antisense nucleotides 354–373) amplifying β_2-microglobulin (Fransen et al., 1994); or GH26-GH27 amplifying the HLA DQα locus (Erlich and Bugawan, 1990). All laboratories except one used at least three HIV-1 specific primer sets. Four laboratories used all three nested Simmonds primer sets: *gag* (gag881, 882, 883 and 990), *pol* (pol001, 002, 003 and 004) and *env* (env401, 402, 403 and 404) (Simmonds et al., 1990). The three SK primer sets SK29-30 with SK31 (LTR region), SK38-39 with SK29 (*gag* region), SK68-69 with SK70 (*env* region) were each used by three laboratories (Ou et al., 1988), one laboratory used SK145-431 with SK102 (Kellogg and Kwok, 1990). One laboratory used the repliprimer set (*pol* region, developed by DuPont): 5′ repliprimer 5′-CCAGGAAGATGGAAACCAAA-3′ (sense nucleotides 2366–2385 of HXB2), 3′ repliprimer 5′-GTCAATGGCCATTGTTTAAC-3′ (antisense nucleotides 2609–2628) with repliprobe 5′-CTGTCTTACTTTGATAAAAC-CTCC-3′ (antisense nucleotides 2402–2425) (Balzarini et al., 1992). Two laboratories developed their own primer sets using the consensus sequence from the Los Alamos HIV Data bank (Myers et al., 1992). LTR *gag* region: primers AV10 5′-TGTGACTCTGGTAACTAGAGATCCCTCAGA-3′ (sense nucleotides 574–603 of HXB2, AC K03455), AV11 5′-TCTAGCAGTGGCGCC-3′ (sense nucleotides 628–642 of HXB2), AV12 5′-GACGCTCTCGCACCC-3′ (antisense nucleotides 791–805 of HXB2), and AV13 5′-CTGCGAATCGTTCTAGCTCCCTGCTTGCCC-3′ (antisense nucleotides 895–924 of HXB2) (Vandamme, 1994). In QC Nos. 1, 2 and 3, AV12 was used as probe for the outer PCR, in QC No. 4, the nested protocol was performed. *pol* region: the nested set H_1POL4235 5′-CCCTACAATCCCCAAAGTCAAGG-3′ (sense nucleotides 4235–4257 of LAI, AC K02013), H_1POL4538 5′-TACTGCCCCTTCAC-CTTTCCA-3′ (antisense nucleotides 4538–4558 of LAI), H_1POL4327 5′-TAAGA-CAGCAGTACAAATGGCAG-3′ (sense nucleotides 4327–4349 of LAI), H_1POL4481 5′-GCTGTCCCTGTAATAAACCCG-3′ (antisense nucleotides 4481–4501 of LAI) (Fransen et al., 1994), occasionally this primer set was used by another laboratory to test its performance. For QC No. 4 *env* region: the nested set H1ENV5944 5′-GCAACCAC-CACTCTCTATTTTGTGC-3′ (sense nucleotides 5944–5968 of LAI), H1ENV6154 5′-AGAGTGGGGTTAATTTTACACATGG-3′ (antisense nucleotides 6154–6178 of LAI), H1ENV6009 5′-GGCCACACATGCCTGTGTAC-3′ (sense nucleotides 6009–6028 of LAI), H1ENV6135 5′-GGCTTTAGGCTTTGATCCCAT-3′ (antisense nucleotides 6135–6155 of LAI) (Fransen et al., 1994) and the nested primer set AV18 5′-GCACCCACCAAGGCAAAGAGAAGAGTGGTG-3′ (sense nucleotides 7712–7741 of HXB2), AV19 5′-AGGAAGCACTATGGGC-3′ (sense nucleotides 7801–7816 of HXB2), AV20 5′-GCTGCTTGATGCCCCA-3′ (antisense nucleotides 7934–7949 of HXB2) and AV21 5′-TTCCACAGCCAGGACTCTTGCCTGGAGCTG-3′ (antisense nucleotides 7946–7975 of HXB2) (Vandamme, 1994) was used.

PCR conditions and analysis of the samples

DNA or lysate (from about 10^5 cells) was amplified in a 50 or 100 μl reaction volume. Amplified PCR samples were stored at −20°C. HIV-1 specific amplification product of a single PCR was detected using a liquid hybridisation protocol (Ehrlich et al., 1990): a [γ-^{32}P]ATP-labelled probe was added to an aliquot of the PCR product and NaCl was added to a final concentration of 100 mM, the mixture was denatured during 10 min at 95°C, hybridised during 1 h at 50–55°C and analysed on an agarose or polyacrylamide gel. The gel was dried or sealed in a plastic bag and autoradiographed at −70°C. Two laboratories performed a Southern blot instead of the liquid hybridisation (Sambrook et al., 1989). In the nested protocol, 1–2 μl of the outer product was transferred to the inner PCR. The amplification product of an inner PCR and the amplified product of the PCR used to check the quality of the DNA was visualised on an agarose or polyacrylamide gel by EtBr staining.

Testing algorithm

The quality of the DNA was checked with a primer set amplifying a one-copy human gene. Three (or more) HIV-1 primer sets were tested at least once. A HIV-1 PCR test was reported positive when at least two of the different HIV-1 PCR assays were positive, the report was negative when all HIV-1 PCR assays were negative and the human gene PCR was positive. All other combinations were reported indeterminate. In one laboratory, a sample that was negative with two of the four primer sets was reported indeterminate. Another laboratory only tested two primer sets, any discordant results between the two primer sets were reported indeterminate. For a positive report, irreproducible results were not taken into account.

Results

3.1. Performance of the Belgian AIDS Reference Laboratories on the MRC reference reagents for a diagnostic HIV-1 DNA PCR

The MRC reference reagents do not contain patient samples and are designed to evaluate the DNA "carryover" problem and frequency of false negative results and to assess inter-laboratory variation of PCR sensitivity due to differences in the detection limit (Bootman and Kitchin, 1992) The results of the MRC quality control No. 1 are given in Table 1. With their in-house protocol, all laboratories correctly reported the samples containing 10 or more copies of HIV-1 DNA. Using the MRC protocol, samples containing 10 or more copies were correctly reported positive by seven of the eight laboratories, only one laboratory reported sample E_1 (10 copies) as indeterminate. No false positive results were reported. Since all positive samples were dilutions from plasmid HIV-1 DNA in negative carrier DNA, the false negative and indeterminate reports were mainly caused by diluted samples, at the border of or below the detection limit. Sample C_1 (0.1 copy) is an obvious example: this sample was tested in 40 experimental settings and was tested positive twice, once with the pol001-004 primer set, and once with the env401-404 primer set, giving rise to two indeterminate reports.

Table 1
Performance of the Belgian AIDS Reference Laboratories on the MRC HIV-1 PCR quality control

Sample	Template molecules of HIV-1 DNA diluted in carrier DNA	DNA quality PCR (all labs)	Report of the laboratories					
			positive		negative		interdeterminate	
			MRC protocol	in-house protocol	MRC protocol	in-house protocol	MRC protocol	in-house protocol
A_1	carrier DNA	+			all labs	all labs		
B_1	1000	+	all labs	all labs				
C_1	0.1	+			7 labs	6 labs	1 lab	1 lab
D_1	1.0	+	3 labs	5 labs	3 labs	1 lab	2 labs	1 lab
E_1	10	+	7 labs	all labs			1 lab	
F_1	100	+	all labs	all labs				
G_1	H_2O	−			all labs	all labs		
H_1	1000	+	all labs	all labs				
I_1	10000	+	all labs	all labs				
J_1	H_2O	−			all labs	all labs		

Laboratory G did not perform the in-house protocol on the MRC samples.
The detection method with the MRC protocol was lab-dependent.

Sample D_1 (1 copy) was also tested in 40 settings, with 20 positive results and 20 times negative or discordant results, giving rise to the false negative and indeterminate reports, which are more or less evenly distributed among the different laboratories.

3.2. Performance of the laboratories on a set of 17 patient samples collected in Belgium and on three reference samples (quality control Nos. 2 and 3)

The results of quality control Nos. 2 and 3 are shown in Table 2. No false positives were reported by any laboratory. Six laboratories reported sample I_3 negative. This sample was a dilution of cDNA obtained from Hut78-HIV_{IIIB} culture supernatant and was below the detection limit of most primer sets. Only laboratory B reported this

Table 2
Performance of the Belgian AIDS Reference Laboratories on the HIV-1 PCR quality control No. 2 and No. 3

Laboratory	Reported positives on 12 true positives (sensitivity)	Reported negatives on 8 true negatives (specificity)	False positives on 8 true negatives	False negatives on 12 true positives	Indeterminate reports on 20 samples
A	10	8	0	1	1
B	12	5	0	0	3
C	9	7	0	1	3
D	11	8	0	1	0
E	9	7	0	1	3
F	8	7	0	1	4
G	11	8	0	1	0
H [a]	7 of 8	3 of 4	0 of 4	0 of 8	2 of 12

[a] Laboratory H did not perform quality control No. 2.

sample positive, they had tested it negative with SK29-30 and the H1POL4235-4538 primers, and positive with SK38-39 and SK68-69 primers. Laboratory H tested it positive only with the primer set gag881-882 and reported I_3 as indeterminate. All other tests for this sample were negative, including these same three primer sets in the other laboratories. Except for I_3, which was not a patient sample, false positive or false negative results were not reported. The indeterminate results were due to failure of some primer sets. Failure of the SK38-39 primer set, used in 60 settings, was involved in 10 of the 15 indeterminate reports: five times on a positive sample and five times on a negative sample. The SK68-69 (used in 57 experiments) and gag881-882 (68 experiments) primer sets were each involved in three indeterminate reports (each once on a negative sample). The three indeterminate reports of laboratory B were all caused by a very weak signal of the SK38-39 primer set with negative samples. Again in the three indeterminate reports of laboratory E, the SK38-39 primer pair was involved. This was also the case with the four indeterminate reports of laboratory F, although the main cause of indeterminate results at that lab was the testing algorithm. With only two primer pairs to rely on (one of them being SK38-39), any discordant result with one primer pair causes an indeterminate report. For laboratory C, the three indeterminate results all involved the primer sets 881-882 giving two times a false negative PCR (together with another primer set) and once a false positive PCR.

3.3. Performance of the different primer sets on the patient samples collected in Belgium

An important difference was noted between the efficacy of the different primer sets to amplify patient-derived HIV strains, collected in Belgian hospitals. The repliprimer set, originally developed by DuPont Inc., performed worst: only three of the nine HIV-1 patient samples were recognised unambiguously (Table 3), five other samples were tested false negative. With the SK38-39, SK68-69 and gag881-882 primers only five of the nine HIV-1 samples were consistently positive among the different laboratories, moreover the SK38-39 and gag881-882 primer sets each failed to detect one of the nine HIV-1 positives in all laboratories (three for SK38-39 and four for gag881-882) using these primer sets. In both cases it was a HIV-1 strain of African origin. None of the other primer pairs repeatedly failed for one of the patient samples, although all other primer pairs, except for the H1POL4235-4538 set, occasionally missed a HIV-1 positive sample, giving rise to discordant results between the different laboratories. False positive results were not found with any primer set, although for the SK primers (which have been used extensively in the labs involved) some true negatives were tested positive in some labs, but this was not reproducible and therefore possibly due to contamination. In some cases, negative samples were not tested negative with some primers when the liquid hybridisation detection was used, due to unusual and probably non-specific bands (in Table 3 classified as unclear results). The nested sets always gave clear specific bands.

Only the H1POL4235-4538 primer set developed by one of the participating laboratories had a 100% sensitivity and 100% specificity but was only tested by this laboratory on nine of the patient samples from QC No. 3 of which six were also tested by a second laboratory. This primer set was selected to be evaluated as a reference primer set in a fourth quality control.

Table 3
Cumulated performance of the primers (and probes) on the patient samples

Primers [a]		Tested positives on 9 true positives (sensitivity)	Tested negatives on 8 true negatives (specificity)	Tested false positives on 8 true negatives	Tested false negatives on 9 true positives	Discordances between the labs, unclear or unreproducible results (on 17 samples)
LTR	SK29-30/SK31 (3)	8	8	0	0	1
GAG	AV10-13/AV12 (1)	8	7	0	0	2
	gag881-882/883-990 (4)	5	7	0	1	4
	SK145-431/SK102 (1)	8	6/6	0/6	0	1/15
	SK38-39/SK19 (3)	5	4	0	1	7
POL	repliprimers/repliprobe (1)	3	6	0	5	3
	pol001-004/002-003 (4)	7	7	0	0	3
	H1POL4235-4538 (2)	5/5	4/4	0/4	0/5	0/9
ENV	env401-404/402-403 (4)	6	8	0	0	3
	SK68-69/SK70 (3)	5	5	0	0	7

Tested positives and tested negatives: samples that tested positive respectively negative in all labs using the specified primer set.
Samples were from quality control Nos. 2 and 3 but I_3, J_3 and K_3 (diluted HIV_{IIIB} cDNA) were omitted in this analysis.
[a] In parentheses, the number of labs that used these PCR primers for their in-house protocol.

3.4. Evalution of the different test algorithms

The test algorithm of the general procedure performed well. When evaluating the test reports of the different laboratories, most mistakes on positive samples were due to the failure of some particular primer sets as discussed above. In samples C_1, D_1 and I_3 the false negative and indeterminate results were due to the dilution of the sample: statistically, not all tests contained one copy of HIV-1 DNA. On several occasions, laboratory F reported a positive sample indeterminate, because only two primer sets were used. If one of the primer sets failed or gave unreproducible amplifications, the sample was reported indeterminate. On two occasions, laboratory C reported a positive sample indeterminate when two assays were positive and two assays were negative.

3.5. Performance of the laboratories when using a standardised protocol

After evaluation of the QC Nos. 1, 2 and 3, a consensus was reached to abandon the worst performing primer pairs and to choose a reliable reference primer set to be used by all laboratories. The fourth quality control was designed to evaluate the performance of the H1POL4235-4538 primers as a possible reference set instead of each laboratory's regular *pol* primer set. Most labs also preferred a nested protocol instead of their previously used liquid hybridisation technique. Laboratories A and B therefore switched to their own nested primer sets (see Materials and methods). The laboratories agreed on the following algorithm: the H1POL4235-4538 reference primers and one or two

lab-dependent primer sets will be used. A sample is reported positive whenever two different primer sets give reproducible positive results. A sample is reported negative when all primer sets test negative and the PCR to check the quality of the DNA is positive. All other combinations result in an indeterminate report. With this algorithm, all laboratories reported the seven HIV-1 positive samples as positive and the three seronegative samples as negative. False positives, false negatives or indeterminate results were not reported in this QC No. 4.

Discussion

This study describes the combined efforts of the eight Belgian AIDS Reference Laboratories to evaluate the performance of their diagnostic HIV-1 DNA PCR on a set of samples distributed among the laboratories in four separate quality controls (QCs).

The quality control of the British Medical Research Council (MRC) (Bootman and Kitchin, 1992) was carried out to assure that the methods used in the different laboratories properly identified positive and negative samples, at an acceptable detection limit (one to five copies of HIV-1 DNA per PCR test). Using their in-house protocol and primers, all laboratories correctly identified the HIV-1 negative samples and the diluted HIV-1 template down to a dilution of 10 copies per PCR test. With the in-house protocol, the one-copy sample was tested positive by five laboratories, indeterminate by one laboratory and negative by another laboratory. This is acceptable since according to the Poisson distribution of targets in solution, statistically the probability of a dropout for a one copy sample is 40% (Greenfield and White, 1993). The detection limit the laboratories is therefore compatible with one to two copies per PCR reaction. Using the MRC primers and conditions, the one-copy sample was tested positive by three laboratories, indeterminate by two laboratories and negative by three laboratories. The slightly lower performance compared to the in-house protocol could be due to the fact that the MRC protocol was simply adapted and not optimised in the different labs. Moreover, the MRC reference kit only provided the outer primers of the three nested sets developed by Simmonds et al. (1990). In some labs, the amplification product was detected using a probe, others used the Simmonds inner primer set or just identified the single PCR product on a EtBr-stained gel. As could be expected, the dilution of 0.1 copy (sample C_1) was reported negative by most laboratories. Again, it is statistically possible that from the 40 experimental settings, two PCR assays contained one copy of HIV-1 DNA and tested positive, giving rise to two indeterminate results.

The second and third QC were established to check the sensitivity and specificity of the different laboratories on Belgian and African samples collected in Belgian hospitals. For historical reasons, a substantial part of the HIV-positive patients seen in Belgian hospitals have African connections. It is therefore important that divergent African strains should not be missed with the primers used. These two quality controls showed that no false positives were reported by any laboratory, suggesting that the laboratories in general did not suffer from PCR carry-over contaminations, although in a few cases of unclear or unreproducible results with some primers, carry-over contamination could not be ruled out. This stresses the continuing need for extremely careful lab practices

(Kwok and Higuchi, 1989). False negatives were only reported in the case of the I_3 sample containing extremely diluted HIV-1 cDNA. Since only one laboratory had reported the MRC one-copy sample negative (with the possibility that this was a true negative due to a stochastic distribution of the one copy), we assume that the I_3 sample contained HIV-1 cDNA below the detection limit of one copy per PCR reaction. All other positive samples containing HIV-1 DNA at a concentration that was above the detection limit of one to two copies were reported positive or indeterminate.

The large amount of indeterminate reports was in most cases due to the combination of primer sets that did not perform satisfactorily on Belgian samples. Since the SK38-39 and SK68-69 primer sets performed poorly, all laboratories that relied on the three frequently used SK primer sets had a high rate of indeterminate reports. The gag881-882 primer set of Simmonds et al. (1990), used by four laboratories, did not perform well, whereas the two other Simmonds primer sets performed rather well. One laboratory only used two primer sets for the detection of HIV-1 DNA, one of them being the poorly performing SK38-39 primer set. Since discordance between the two primer sets resulted in an indeterminate report, this laboratory had the largest amount of indeterminate reports.

The laboratories concluded that in Belgium at least one reliable common primer set was needed. Laboratory B had developed new *pol* primers situated within very conserved regions as judged from the most recent Los Alamos consensus HIV-1 sequence (Myers et al., 1992) (including many African sequences). These primers already proved reliable on Belgian and African patients (Fransen et al., 1994). Additionally, the nine patient samples of QC No. 3 were all tested correctly with this H1POL4235-4538 nested primer set. All laboratories agreed to set up a new QC No. 4 to test its reliability as a Belgian reference primer set.

The laboratories agreed on the following algorithm: (1) A positive PCR test with two different primer sets is needed for a positive report. Two primer sets are initially tested (one of them being the *pol* reference primer set) and a third primer set is used to confirm a positive result with only one of the two initial primer sets.

(2) A negative report involves negative test results for all HIV specific primer sets and a positive test for the human DNA PCR.

(3) All other combinations are reported indeterminate.

In QC No. 4, all laboratories attained a sensitivity and a specificity of 100% (on 10 patient samples). The H1POL4235-4538 nested primer set was accepted as the Belgian reference primer set for the detection of HIV-1 DNA and the tested algorithm is now used by all laboratories. By choosing only one reference primer set to be used in common, the laboratories retained flexibility in their choice of second and third primer sets. In this way the reference laboratories have the possibility to send each other indeterminate samples to be tested with additional primer sets.

In this multicentre quality control, the eight Belgian AIDS Reference Laboratories have been shown to be able to detect one to two copies of HIV-1 DNA per PCR test. As a result of these quality controls, they have improved their performance by abandoning primers with an unacceptable performance on Belgian patients, by accepting a reliable reference primer set and by adapting a reliable general test algorithm. This study thus resulted in the standardisation of the diagnostic HIV-1 DNA PCR in Belgium. Yearly

quality controls will continue to assure that the choice of the primers and the performance of the laboratories remains satisfactory.

The African samples tested were collected in Belgian hospitals and were derived from patients originating from different African countries. Since the genomic variability among HIV-1 strains is largest in Africa (Louwagie et al., 1993), the primers developed using the Los Alamos consensus sequences and optimised for the detection of HIV-1 DNA from patients in Belgium will be of benefit for all centres that receive samples of diverse geographical origin.

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