

Cervicovaginal anti-HIV antibodies in HIV-seronegative female sex workers in Abidjan, Côte d'Ivoire

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Objective: To detect anti-HIV antibodies in cervicovaginal secretions of HIV-seronegative female sex workers and to evaluate whether the presence of these antibodies is associated with increased sexual exposure.

Methods: A cross-sectional study was carried out at a confidential clinic for female sex workers in Abidjan, Côte d'Ivoire. The participants were 342 HIV-seronegative female sex workers in whom a cervicovaginal lavage was collected. The main outcome measures were the detection of antibodies to HIV-1 in cervicovaginal lavages using an in-house and a commercial (Seradyn Sentinel[®]; Calypte Biomedical Corporation, Berkeley, California, USA) enzyme immunoassay; the detection of semen in cervicovaginal lavages; and the assessment of epidemiological and biological markers of sexual exposure to HIV.

Results: Cervicovaginal anti-HIV antibodies were detected in 7.3 and 29.8% of women using in-house enzyme-linked immunosorbent assay (ELISA) and Seradyn Sentinel respectively. All cervicovaginal secretions found to be positive by in-house ELISA were also positive by Seradyn Sentinel. In a minority of women, ranging from 2.9% by in-house ELISA to 12.3% by Seradyn Sentinel, the anti-HIV antibodies were present in vaginal fluids that did not contain semen. Sexual exposure to HIV was similar in women with anti-HIV antibodies in their semen-free cervicovaginal secretions compared with women without anti-HIV antibodies in their cervicovaginal secretions.

Conclusions: Cervicovaginal HIV-specific antibodies were detected in a minority of sexually exposed HIV-seronegative female sex workers in Abidjan. The lack of association between increased sexual exposure to HIV and presence of cervicovaginal HIV-specific antibodies suggests that the production of genital HIV-specific antibodies in exposed seronegative women depends on the ability of individual women to mount specific mucosal immunity to HIV antigens, the determinants of which are currently unknown.

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AIDS 2000, **14**:2603–2608

Keywords: Africa, HIV resistance, female sex workers, mucosal immunity

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Note: The use of trade names and commercial sources is for identification only and does not imply endorsement by the Public Health Service or the US Department of Health and Human Services.

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Received: 9 September 1999; revised: 14 June 2000; accepted: 28 June 2000.

Introduction

A small proportion of individuals remain HIV-seronegative despite repeated sexual exposure to the virus, suggesting that they may be resistant to HIV infection [1–4]. Several mechanisms of HIV-1-resistance have been proposed, including circulating HIV-1-specific cytotoxic T-lymphocyte immune responses [3] and a defective CCR5 receptor [5,6], although this genetic defect has not been reported in African populations [6,7].

Mucosal immunity may also play a role in resistance to HIV infection. HIV-specific antibodies of the immunoglobulin IgA or IgG isotypes have been detected in cervicovaginal secretions of exposed HIV-seronegative women [8–10] and of HIV-seronegative female partners of HIV-seropositive male individuals in sero-discordant couples [11]. Recent reports have also demonstrated the presence of HIV-1-specific IgA in the genital tract of highly exposed persistently HIV-1-seronegative Kenyan [12] and Thai [13] sex workers.

The objectives of the present study were to detect HIV-specific antibodies in cervicovaginal secretions of HIV-seronegative female sex workers and to evaluate whether the presence of cervicovaginal antibodies to HIV is associated with increased sexual exposure in these women.

Methods

Study population and processing of clinical samples

A cross-sectional study was conducted from April 1994 to November 1995 at a confidential clinic for female sex workers in Abidjan, as reported earlier [14]. Briefly, female sex workers were interviewed and a gynaecological examination was performed. Genital ulcers were diagnosed clinically and laboratory tests were performed for the detection of *Trichomonas vaginalis*, *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. A wet mount preparation of vaginal secretions collected from the posterior vaginal fornix was examined for the presence of motile *T. vaginalis*. Endocervical swabs were collected for the detection of *C. trachomatis* by enzyme immunoassay (EIA Microtrak; Syva Co, Palo Alto, California, USA) and for culture of *N. gonorrhoeae* on modified Thayer–Martin medium. Samples positive for *C. trachomatis* by enzyme immunoassay were confirmed by a blocking assay.

Peripheral blood samples were collected for HIV and syphilis serology and for HIV RNA detection. Cervicovaginal secretions were collected by a standardized vaginal lavage with 10 ml of phosphate-buffered saline

(PBS). The cervicovaginal lavages were centrifuged at 1000 g for 10 min, and the supernatants were kept frozen at -80°C until processing. The lavage procedure corresponds to a 1 : 30-dilution of the cervicovaginal secretions [15].

Serum samples were screened for antibodies to HIV-1 and HIV-2 by a second-generation enzyme-linked immunosorbent assay (ELISA) (Genelavia Mix; Sanofi-Diagnostics Pasteur, Marnes-la-Coquette, France). Positive samples were further confirmed using an algorithm including a synthetic peptide-based test (Peptilav 1-2; Sanofi-Diagnostics Pasteur) and Western blot (New-LAV Blot I; Sanofi-Diagnostics Pasteur and HIV Blot 2.2; Diagnostic Biotechnology Ltd, Geneva, Switzerland). Antitreponemal antibodies were detected by *Treponema pallidum* haemagglutination assay (TPHA; Fujirebio, Tokyo, Japan). HIV-1 RNA was detected in serum by an in-house reverse transcription (RT)-nested polymerase chain reaction (PCR) for gp41 *env*-encoded gene using primers adapted to detect African clades [16].

Antibodies to HIV in cervicovaginal secretions

Total IgA+IgG+IgM antibodies to gp160 were detected in 100 μl of cervicovaginal lavage by in-house indirect ELISA, using the biotine-streptavidine system for revelation [17]. The gp160 antigen consisted of a purified preparation of baculovirus-expressed recombinant gp160 (rgp160), derived from the envelope of the LAI strain of HIV-1 (Transgène, Strasbourg, France). This assay detects all molecular forms of IgA and IgM present in body fluids (monomeric as well as polymeric immunoglobulin), and IgG. Wells of polyvinyl chloride microtitre plates (Nunc, Kampstrup, Denmark) were coated overnight at 37°C with 100 μl of an optimal concentration (1 $\mu\text{g}/\text{ml}$) of rgp160 using PBS as coating buffer. Excess antigen was removed and the plates were washed four times with PBS-Tween 20 and blocked with 100 μl of 2.5% (wt/vol) skim milk powder in PBS for 1 h at 37°C . One hundred microlitres of cervicovaginal washing were incubated overnight at 4°C . After five washings with PBS-Tween 20, 100 μl of goat biotinylated antibodies to human immunoglobulin (Sigma Chemical Co., St Louis, Missouri, USA), diluted 1 : 2000 in PBS, were added at 37°C . After 1 h incubation and five washings with PBS-Tween 20, 100 μl of streptavidine horseradish peroxidase conjugate (Sigma Chemical Co.), diluted 1 : 1000 in PBS, were incubated for 30 min at 37°C . Peroxidase activity was read at 492 nm. For each tested sample, a paired well without antigen, only coated with blocking solution, served as negative control. This control increased the specificity of the assay since some cervicovaginal components can non-specifically bind immunoglobulins to the solid phase [18]. Results were given as the difference in optical density between the antigen-coated and the antigen-free control wells.

Anti-HIV antibodies were also detected in 100 μ l cervicovaginal lavages by a commercial enzyme immunoassay proposed for use on urine and other body fluid samples (Seradyn Sentinel[®] HIV-1 Urine EIA; Calypte Biomedical Corporation, Berkeley, California, USA), according to the manufacturer's instructions. The Seradyn Sentinel assay detects HIV-specific IgG and IgM, but not IgA. The coating antigen is a baculovirus-expressed complete gp160 protein. Although this assay is considered to be highly sensitive to detect HIV-specific antibodies in body fluids, especially in urine [19], it has a false-positive rate of about 1% in urine in low risk populations (package insert data).

For both ELISAs the cutoff of positivity for cervicovaginal secretions was calculated as the mean plus two standard deviations of the reactivities obtained by testing 100 μ l of the cervicovaginal lavages obtained from 30 healthy HIV-seronegative Caucasian women not at risk for HIV. Fifteen cervicovaginal fluids from HIV-1-seropositive women were used as positive controls.

Detection of contaminating semen in cervicovaginal secretions

Two semen markers, prostatic specific antigen (PSA) and prostatic acid phosphatase (PAP), were detected in the supernatant of cervicovaginal secretions in which anti-HIV antibodies were detected. Commercially available immuno-enzymatic tests exhibiting a threshold of positivity of 0.1 ng/ml according to the manufacturer (PSA and PAP IMX[®] System; Abbott Laboratories, Abbott Park, Chicago, Illinois, USA) were used. The cutoffs of positivity for PSA and PAP antigens in cervicovaginal fluid were determined as the mean plus two standard deviations of the values obtained with a 1:30 dilution in PBS of cervicovaginal secretions obtained from 30 healthy childbearing-aged HIV-seronegative Caucasian women who had not had sexual intercourse for at least 5 days; for both markers, the cutoff was 0.4 ng/ml. One to 10 repeated freeze-thawing cycles did not affect the performance of PSA and PAP assays (data not shown). Lack of contaminating semen in cervicovaginal secretions was defined as lack of detectable PSA or PAP in cervicovaginal secretions; contamination of cervicovaginal secretions with semen was defined by the presence of at least one of these markers.

Statistical analysis

Frequencies of women with anti-HIV antibodies are given for each of the assays used to detect anti-HIV antibodies. Among the cervicovaginal lavages with anti-HIV antibodies, the proportion that did not contain markers of semen is given. The presence of markers of sexual exposure including selected sexually transmitted diseases was compared between women with anti-HIV antibodies but no semen in their cervicovaginal lavages and women without anti-HIV

antibodies in their lavages. Women with both anti-HIV antibodies and semen in their cervicovaginal secretions were excluded from this analysis. Indeed, since the seminal fluid of HIV-infected men contains IgG antibodies to HIV [20], it was not possible to discriminate in these women whether the anti-HIV antibodies detected in cervicovaginal lavages were produced by the female genital tract or were heterologous antibodies from the semen of an HIV-infected male partner. Comparisons were tested for statistical significance with a 2×2 table continuity adjusted χ^2 test or Fisher's exact test or a χ^2 test for linear trend.

Results

A total of 1201 female sex workers were evaluated during the study period. Among the 1079 sex workers who consented to HIV testing, 657 (61%) were positive including 40% HIV-1 seropositive, 2% HIV-2 seropositive and 19% dually seroreactive to HIV-1 and HIV-2. Of the 420 HIV-seronegative women, vaginal lavages were collected in 342 women (81%). The median age of these women was 26 years and their median duration of sex work was 24 months. During their last working day, 43% of them had used condoms with all their clients. Among these 342 women 27% had trichomoniasis, 20% had a positive TPHA test, 14% had gonorrhoea, 5% had a chlamydial infection, and 3% showed a genital ulcer.

Cervicovaginal HIV-specific antibodies in HIV-seronegative women

By in-house ELISA, the cervicovaginal fluid of 25 women (7.3%) tested positive for anti-HIV antibodies, 10 (2.9%) of which did not contain semen. The mean optical density (OD) at 492 nm obtained with the 10 semen-free cervicovaginal secretions positive by the in-house ELISA (mean \pm SD, 0.531 ± 0.446 ; range, 0.217–1.71) was 6.2-fold higher than the mean OD obtained with the cervicovaginal secretions from the 30 HIV-seronegative healthy controls (0.085 ± 0.031 ; range, 0.012–0.097), and 5.1-fold lower than the mean OD obtained with the 15 cervicovaginal secretions from HIV-positive controls (2.93 ± 0.16 ; range, 2.50–3.00). By Seradyn Sentinel HIV-1 Urine EIA the cervicovaginal fluid of 102 women (29.8%) tested positive, 42 (12.3%) of which did not contain semen. The mean OD at 450 nm obtained with the 42 semen-free cervicovaginal secretions positive by the Seradyn Sentinel HIV-1 Urine EIA (0.773 ± 0.489 ; range, 0.355–3.00) was 3.6-fold higher than the mean OD obtained with the cervicovaginal secretions from the 30 HIV-seronegative healthy controls (0.217 ± 0.062 ; range, 0.041–0.230), and 3.8-fold lower than the mean OD obtained with the 15 cervicovaginal secretions from HIV-positive controls (2.95 ± 0.13 ; range, 2.57–

3.00). All cervicovaginal secretions positive by the in-house ELISA were also positive by Seradyn Sentinel HIV-1 Urine EIA. All cervicovaginal secretions from HIV-infected women were positive by both assays.

The 25 women with anti-HIV antibodies in their cervicovaginal secretions by both ELISAs were confirmed to be HIV-uninfected by HIV-1 RNA RT-nested PCR in plasma.

Relationship between sexual exposure and cervicovaginal HIV-specific antibodies

Table 1 compares socio-demographic and behavioral factors, and STD and genital ulcer in women without anti-HIV antibodies in their cervicovaginal secretions and in women with anti-HIV antibodies by in-house ELISA in their semen-free cervicovaginal secretions, as assessed by both ELISAs. For both comparisons there were no differences between the two groups in age, duration of sex work, frequency of condom use, number of clients, and prevalences of gonorrhoea, chlamydial infection, trichomoniasis, positive TPHA test, or genital ulcer.

Discussion

Antibodies directed to the *env*-encoded surface glycoprotein gp160 were detected in the cervicovaginal secretions of a small proportion of HIV-seronegative sex workers in Abidjan. In 2.9 to 12.3% of these women, depending on the test used, the anti-HIV antibodies were present in vaginal fluids that were free of contaminating semen. Since there is no established gold standard test, it is unclear which of these two proportions is the best estimate of the real prevalence rate of cervicovaginal anti-HIV antibodies in the absence of contaminating semen in HIV seronegative sex workers. The presence of PAP and PSA in more than half of the HIV-seronegative women with cervicovaginal anti-HIV antibodies demonstrates that these women had unprotected sex and emphasizes the requirement for the search of evidence of contaminating semen when investigating local immunity in exposed women.

We chose to use in parallel our in-house ELISA and the commercially available Seradyn Sentinel HIV-1 Urine EIA. The in-house ELISA is highly specific to detect IgA, IgG and IgM antibodies to gp160 in body

Table 1. Markers of sexual exposure in female sex workers with anti-HIV antibodies in their semen-free cervicovaginal secretions, and in women without anti-HIV antibodies in their cervicovaginal secretions, by in-house enzyme-linked immunosorbent assay (ELISA) and Seradyn Sentinel[®] HIV-1 Urine enzyme immunoassay (EIA).

	In-house ELISA			Seradyn Sentinel HIV-1 Urine EIA		
	Ab-pos ^a n = 10	Ab-neg n = 317	<i>P</i> ^c	Ab-pos ^b n = 42	Ab-neg n = 240	<i>P</i> ^c
Socio-demographic and behavioral factors						
Age ≥ 30 ^d	2 (20)	129 (41)	0.3	17 (40)	96 (40)	1
Duration of sex work in months						
< 12	2 (20)	85 (27)	0.7	10 (24)	63 (26)	0.8
12–23	2 (20)	52 (16)		6 (14)	38 (16)	
24–59	6 (60)	106 (33)		18 (43)	87 (36)	
≥ 60	0 (0)	74 (23)		8 (19)	52 (22)	
Inconsistent condom use	8 (80)	266 (84)	0.7	37 (88)	201 (84)	0.6
≥ 5 clients during past week ^d	6 (60)	168 (57)	1	19 (50)	131 (57)	0.5
Sexually transmitted agents and syndrome						
<i>Neisseria gonorrhoeae</i> ^d	1 (10)	46 (15)	1	3 (7)	33 (14)	0.3
<i>Chlamydia trachomatis</i> ^d	0 (0)	14 (5)	1	0 (0)	11 (5)	0.4
<i>Trichomonas vaginalis</i>	4 (40)	84 (27)	0.5	14 (33)	61 (25)	0.3
TPHA positive	0 (0)	63 (20)	0.2	9 (21)	46 (19)	0.9
Genital ulcer	0 (0)	9 (3)	1	1 (2)	5 (2)	1

Data are number (%). TPHA, *Treponema pallidum* haemagglutination assay.

^aSerum HIV RNA polymerase chain reaction negative women with in-house ELISA anti-HIV antibodies but no semen in their cervicovaginal secretions.

^bWomen with Seradyn Sentinel HIV-1 Urine EIA anti-HIV antibodies but no semen in their cervicovaginal secretions.

^cContinuity adjusted 2 × 2 table χ^2 or Fisher's exact test or χ^2 test for linear trend.

^dAge missing for one antibody negative woman; number of clients during past week missing for 20 antibody negative women; *N. gonorrhoeae* result missing for six antibody negative women; *C. trachomatis* result missing for seven antibody negative women, by in-house ELISA. Age missing for one antibody negative woman; number of clients during past week missing for four antibody positive women and 11 antibody negative women; *N. gonorrhoeae* result missing for five antibody negative women; *C. trachomatis* result missing for six antibody negative women by Seradyn Sentinel HIV-1 Urine EIA.

fluids. However this assay may lack sensitivity for cervicovaginal secretions with a low titre of HIV-specific antibodies or for cervicovaginal secretions containing HIV-specific antibodies of low avidity [17,18]. The Seradyn Sentinel HIV-1 Urine EIA, previously used by Mazzoli *et al.* [11] and Kaul *et al.* [12], is considered to be highly sensitive to detect IgG and IgM antibodies to gp160 in body fluids [19], although it may lack specificity for some cervicovaginal samples. Interestingly, Beyrer *et al.* showed that a full length gp160 was necessary to detect IgA in the cervicovaginal secretions of commercial female sex workers in Thailand, whereas a gp120 assay did not work [13]. This finding justifies the use of recombinant gp160 as coating antigen as in our in-house ELISA and in the Seradyn Sentinel HIV-1 Urine EIA assay.

The 25 HIV-1-seronegative sex workers with anti-HIV antibodies in their semen-free cervicovaginal secretions by both in-house ELISA and Seradyn Sentinel HIV-1 Urine EIA had no evidence of HIV-1 RNA in plasma. It is therefore unlikely that these antibodies are part of a primary HIV infection, although these women were not followed up.

In the present study, increased sexual exposure was not associated with the presence of HIV-antibodies in cervicovaginal secretions, as measured by either of the two tests. Similarly, Mazzoli *et al.* did not find an association between the duration of unprotected sex and the presence of HIV-specific antibodies in the urine or cervicovaginal secretions of HIV-seronegative female partners of HIV-seropositive males in sero-discordant couples [11]. A recent study in Nairobi showed that HIV-specific genital antibodies of the IgA isotype were more frequently detected among HIV-uninfected women who had been in sex work for at least 3 years (76%) than among HIV-seronegative women with low risk of HIV infection (11%) [12]. However, this study did not control for the presence of semen in the cervicovaginal secretions, leaving the possibility that some of the IgA anti-HIV antibodies were heterologous antibodies present in the semen of an HIV-infected male partner. A recent study in Northern Thailand also showed that HIV-specific genital antibodies of the IgA isotype were more frequently detected among HIV-uninfected women who had done sex work for at least 2 years and who had two or more documented STD episodes (38%) than among HIV-seronegative women with low risk of HIV infection (0%) [13]. The studies among sex workers in Nairobi and Thailand did not include women who had been exposed for less than 2 years, as ours did. Our study suggests that women need not be exposed to HIV over several years to produce genital antibodies to HIV. It further suggests that only some women may have the capacity to produce genital HIV-specific antibodies in response to an

exposure to HIV, the determinants of which are currently unknown.

In conclusion, cervicovaginal HIV-specific antibodies were present in a small proportion of sexually exposed HIV-seronegative female sex workers in Abidjan, and were not related to increased sexual exposure. Prospective studies are needed to determine whether these mucosal anti-HIV antibodies are consistently present and confer protection against sexual transmission of HIV. In the meantime exposed HIV-seronegative female sex workers should continue to be intensively counselled about condom use.

Acknowledgements

We thank the Abidjan female sex worker community for their cooperation. We further thank M. N'Krumah, T. Aka and the peer educators' network for their daily efforts at Clinique de Confiance; Y.M. Hoyi, M.-J. Teurquetil, M.-L. Adom, D. Soroh, D. Yavo and K. Diallo for laboratory support; J.-C. Kouadio and B. Gbaye for data entry and management; and the staff of the 'Programme de Prévention et de Prise en charge des MST/SIDA chez les femmes libres et leur Partenaires' for their collaboration. We are indebted to M. Matta and A. Si-Mohamed for technical contribution or advice. We thank Calypte Biomedical Corporation, Berkeley, California, USA, for providing kits for the study.

Sponsorship: The study was supported in part by grants from the European Community Programme on Science and Technology for Development, Brussels, from the Nationaal Fonds voor Wetenschappelijk Onderzoek, Brussels, and from the Agence Nationale de Recherches sur le SIDA (France). P.D.G. was a scholar of the American Foundation for AIDS Research.

References

1. Clerici M, Giorgi JV, Chou C-C, *et al.* **Cell-mediated immune response to human immunodeficiency virus (HIV) type 1 in seronegative homosexual men with recent sexual exposure to HIV-1.** *J Infect Dis* 1992, **165**:1012-1019.
2. Detels R, Liu Z, Hennessey K, *et al.* **Resistance to HIV-1 infection.** *J Acquir Immune Defic Syndr* 1994, **7**:1263-1269.
3. Rowland-Jones S, Sutton J, Ariyoshi K, *et al.* **HIV-specific cytotoxic T-cells in HIV-exposed but uninfected Gambian women.** *Nature Med* 1995, **1**:59-64.
4. Fowke KR, Nagelkerke NJD, Kimani J, *et al.* **Resistance to HIV-1 infection among persistently seronegative prostitutes in Nairobi, Kenya.** *Lancet* 1996, **348**:1347-1351.
5. Liu R, Paxton WA, Choe S, *et al.* **Homozygous defect in HIV-1 coreceptor accounts for resistance of some multiply-exposed individuals to HIV-1 infection.** *Cell* 1996, **86**:367-377.
6. Samson M, Libert F, Dorantz BJ, *et al.* **Resistance to HIV-1 infection of caucasian individuals bearing mutant alleles of the CCR5 chemokine receptor gene.** *Nature* 1996, **382**:722-725.
7. Koblavi S, Nkengasong J, Maurice C, *et al.* **Immune activation**

- markers and chemokine receptor 5 (CCR5) genotype in highly exposed but persistently seronegative female sex workers in Abidjan, Côte d'Ivoire. *XII International Conference on AIDS*, Geneva, June–July 1998 [abstract 31147].
8. Bélec L, Grésenguet G, Dragon M-A, Meillet D, Pillot J. **Detection of antibodies to human immunodeficiency virus in vaginal secretions by immunoglobulin G antibody capture enzyme-linked immunosorbent assay: application to detection of seminal antibodies after sexual intercourse.** *J Clin Microbiol* 1994, **32**:1249–1255.
 9. Bélec L, Tevi-Bénissan C, Grésenguet G, Meillet D, Pillot J. **HIV-1 antibody serum negativity with vaginal secretions positivity.** *Lancet* 1994, **343**:1046–1047.
 10. Bélec L, Matta M, Tevi-Bénissan C, Payan C, Meillet D, Pillot J. **Detection of seminal antibodies to HIV in vaginal secretions after sexual intercourse: a possible tool to prevent the risk of HIV transmission in a rape victim.** *J Med Virol* 1995, **45**: 113–116.
 11. Mazzoli S, Trabattoni D, Caputo SL, et al. **HIV-specific mucosal and cellular immunity in HIV-seronegative partners of HIV-seropositive individuals.** *Nature Med* 1997, **3**:1250–1257.
 12. Kaul R, Trabattoni D, Bwayo JJ, Arienti D, Clerici M, Plummer F. **HIV-1 specific mucosal IgA in a cohort of HIV-1 resistant Kenyan prostitutes.** *AIDS* 1999, **13**:23–29.
 13. Beyrer C, Artenstein AW, Ruggao S, et al. **Epidemiologic and biologic characterization of a cohort of human immunodeficiency virus type 1 highly exposed, persistently seronegative female sex workers in Northern Thailand.** *J Infect Dis* 1999, **179**: 59–67.
 14. Ghys PD, Fransen K, Diallo MO, et al. **The associations between cervicovaginal HIV shedding, sexually transmitted diseases and immunosuppression in female sex workers in Abidjan, Côte d'Ivoire.** *AIDS* 1997, **11**:F85–F93.
 15. Bélec L, Meillet D, Lévy M, Georges A, Tévi-Bénissan C, Pillot J. **Dilution assessment of cervicovaginal secretions obtained by vaginal washing for immunological assays.** *Clin Diag Lab Immunol* 1995, **2**:57–61.
 16. Yang C, Pieniazek D, Owen SM, et al. **Detection of phylogenetically diverse human immunodeficiency virus type 1 groups M and O from plasma by using highly sensitive and specific generic primers.** *J Clin Microbiol* 1999, **37**:2581–2586.
 17. Bélec L, Tevi-Bénissan C, Dupre T, et al. **Comparison of cervicovaginal humoral immunity in clinically asymptomatic (CDC A1 and A2 categories) patients with HIV-1 and HIV-2 infection.** *J Clin Immunol* 1996, **16**: 12–20.
 18. Lu X-S, Bélec L, Pillot J. **High frequency of anti-gp160 IgG and IgA antibodies associated to a large increase of total IgG in cervicovaginal secretions from HIV-1 infected women.** *J Infect Dis* 1993, **167**: 1189–1192.
 19. Urnovitz HB, Sturge JC, Gottfried TD. **Increased sensitivity of HIV-1 antibody detection.** *Nature Med* 1997, **3**:1258.
 20. Wolff H, Mayer K, Seage G, Politch J, Horsburgh CR, Anderson D. **A comparison of HIV1 antibody classes, titers, and specificities in paired semen and blood samples from HIV1 seropositive men.** *J Acquir Immune Defic Syndr* 1992, **5**:65.