

# Fertility and Sterility

Copyright © 2002 American Society for Reproductive Medicine

Volume 78(6)

December 2002

pp 1199-1202

## Comparison of polymerase chain reaction-dependent methods for determining the presence of human immunodeficiency virus and hepatitis C virus in washed sperm

[Male Factor]

Meseguer, Marcos Ph.D.<sup>a,b</sup>; Garrido, Nicolás Ph.D.<sup>a,b</sup>; Gimeno, Concha M.D.<sup>c,e</sup>; Remohí, José M.D.<sup>a,b,d</sup>; Simón, Carlos M.D.<sup>a,b,d</sup>; Pellicer, Antonio M.D.<sup>a,b,d,e</sup>

Instituto Valenciano de Infertilidad Valencia (IVI), Valencia, Spain, and Instituto Valenciano de Microbiología (IVAMI), Valencia, Spain

<sup>a</sup> Instituto Valenciano de Infertilidad Valencia (IVI).

<sup>b</sup> Fundación IVI para el Estudio de la Reproducción, Valencia, Spain.

<sup>c</sup> Instituto Valenciano de Microbiología (IVAMI).

<sup>d</sup> Department of Pediatrics, Obstetrics and Gynecology and Department of Microbiology, Valencia University School of Medicine, Valencia, Spain.

<sup>e</sup> Department of Obstetrics and Gynecology Hospital Dr. Peset, Valencia, Spain.

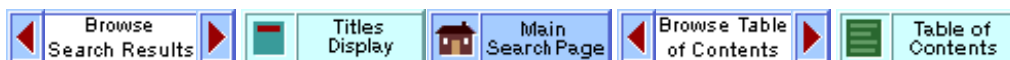
Received January 2, 2002; revised and accepted April 15, 2002.

This work was supported by a grant from the Ministry of Health of the Valencian Government.

Reprint requests: Marcos Meseguer, Ph.D., Instituto Valenciano de Infertilidad, Andrology Laboratory and Semen Bank, Plza. Policia Local 3, 46015 Valencia, Spain (FAX: 34 96 3050999; E-mail: andrologiavalencia@ivi.es).

[PayPerView Account](#)

[View Cart](#) 



### Outline

- [Abstract](#)
- [MATERIALS AND METHODS](#)
  - [Sperm Wash](#)
  - [PCR Techniques for HIV Applied to Semen](#)
- [RESULTS](#)
- [DISCUSSION](#)
- [References](#)

### Graphics

- [Table 1](#)

### Abstract

Objectives: To evaluate the effectiveness of sperm washes by double capacitation in patients with human immunodeficiency virus (HIV) and hepatitis C virus (HCV) and to compare between polymerase chain reaction (PCR)-dependent methods to determine viral presence after the procedure.



#### Output...

[Print Preview](#)  
[Email Article Text](#)  
[Save Article Text](#)  
[Full Text \(PDF\) 599 K](#)

#### Links...

[About this Journal](#)  
[Abstract Complete Reference](#)  
[Help](#)  
[Logoff](#)

#### History...

Comparison of polymerase ...    
[Previous Page](#)

Design: Controlled prospective clinical trial.

Setting: Private and university-affiliated setting.

Patient(s): Thirty-four HIV-positive men attending our center for assisted reproduction with their partners.

Intervention(s): Forty-one semen samples from seropositive males were obtained and washed through density gradients and swim-up and analyzed for the presence of HIV and HCV with either nested or one-round PCR.

Main Outcome Measure(s): Qualitative and quantitative detection of DNA and RNA of HIV and RNA of HCV.

Result(s): Of all the semen samples that were analyzed after washing, seven out of 41 samples tested positive for one or both viruses by nested PCR. The confirmation of these results with the currently used commercial methods of quantitative PCR (one-round PCR) resulted in all negatives.

Conclusion(s): Sperm wash with nested PCR is the appropriate method to use in the assisted reproduction techniques that are offered to serodiscordant couples. The detection limits exhibited by one-round PCR do not offer a sufficient guarantee that transmission of all viral particles will be avoided since both viruses can be present in amounts lower than the detection threshold of this technique.

---

Many serodiscordant couples, that is, couples in which the male is infected with human immunodeficiency virus (HIV) or hepatitis C virus (HCV) and the female is free of these viruses, wish to conceive children. Unfortunately, because of the risk of transmission of virus in semen, these couples should use barrier methods of protection when engaging in sexual intercourse, which eliminates any possibility of achieving pregnancy.

Sexual transmission of HIV is widely recognized, and many studies have demonstrated the presence of the virus mainly in seminal plasma and seminal round cells (1). The HCV situation is more controversial, since many studies are unable to find it even in raw semen (2,3). On the other hand, other studies clearly demonstrate the presence of the HCV viral DNA in raw semen (4,5).

Detection methods are commonly based on the amplification of well-defined sequences of viral nucleic acids. However, the first reports of noninfected females and newborns after the semen washes and artificial insemination (AI) for HIV were published in Italy by using immunofluorescence for p24 antigen, which has a higher detection limit (6), thus showing less sensitivity in comparison with the polymerase chain reaction (PCR) method.

Nevertheless, common PCR procedures, such as those employed to quantify the blood viral load, when employed to determine viral load in washed semen samples may not be enough, since there is a limit to the number of copies that can be detected (7-9).

Another way to determine the presence/absence of viral nucleic acids in postwash spermatozoa is the employment of nested PCR techniques, which decrease the detection limit to one copy of viral RNA/DNA (10).

The aims of our study were to determine the presence or absence of viral load for HIV/HCV viruses after the wash of the samples and, in those cases where positive results were found, to compare the results with one-round quantitative PCR.

## MATERIALS AND METHODS

Thirty-four HIV-1-seropositive men were included in this study, with a total number of 41 samples. All of them signed an informed consent, and the study was approved by the Institutional Review Board of the Instituto Valenciano de Infertilidad.

The patients showed all the stages of the infection (A, B, or C) according to the Centers for Disease Control classification. Thirty-three (97%) of the men were receiving anti-retroviral treatment. The average age was 36 years (range, 25-44 years).

Total count and motility before and after the wash were recorded. Sperm wash was only performed in those samples with more than 2 million total progressive motile sperm in the whole semen sample.

### Sperm Wash

Ejaculates obtained after a sexual abstinence of 3-5 days were allowed to liquefy and then were diluted 1:1 (vol:vol) with sperm medium (MediCult, Jyllinge, Denmark). Then they were pelleted at 400 g for 10 minutes and the supernatants were discarded.

A volume of sperm medium equal to the initial volume was added and then layered onto a triple-density gradient (90%, 70%, and 45%; PureSperm; Nidacon, Goteborg, Sweden) of 1-1.2 mL for each layer and centrifuged for 20 minutes at 300 g.

Each pellet was obtained and washed with 5 mL of sperm medium and pelleted again. Supernatants were discarded, and a swim-up of 0.5-0.7 mL was done. After 45 minutes, the upper 0.35 mL of each tube were obtained and pooled.

One-half was immediately submerged into liquid nitrogen for PCR determination, and the other half was frozen with sperm freezing medium (MediCult) according to the manufacturer's instructions and stored until use after a finding of negative viral presence.

### PCR Techniques for HIV Applied to Semen

Nucleic acids were extracted from the washed spermatozoa using the Nuclisense method (Organon Teknika, Barcelona, Spain) following the instructions of the manufacturer. Two extractions were run in parallel, one from the spermatozoa sample and the other with the spermatozoa samples after the addition of HIV RNA obtained from HIV-infected plasma to detect the presence of transcription or amplification inhibitors after the nucleic acid extraction procedure. Both extracted samples were used for two HIV RNA transcriptions to detect genes from the *gag* and *pol* region, followed by a nested DNA amplification (10). The same samples were used to amplify HIV proviral DNA by a nested amplification to detect both genes (*gag* and *pol*). The other extraction, run in parallel with added HIV RNA before nucleic acid extraction, was used as a positive control to detect the presence of inhibitors of the transcription or amplification. Besides negative controls to detect the presence of amplicons, contamination was carried out.

For HIV RNA transcription, we used the antisense external primers to anneal with nucleotides 1696-1676 and 3286-3265 for the *gag* and *pol* genes, respectively. Standardized conditions for transcription were followed using 100  $\mu$ M DTT, 1 mM each dNTP, 0.2  $\mu$ M antisense primer, 20 U RNAasin (Promega, Barcelona, Spain), and 5 U AMV transcriptase (Promega) in a final volume of 20  $\mu$ L. Nested DNA amplification used the external primers to anneal with nucleotides (from ARV2/SF2 sequence) 1224-1243 and 1696-1676 and internal primers to anneal with nucleotides 1316-1335 and 1524-1504 for the *gag* region. External primers annealing with

nucleotides 2623-2642 and 3286-3265 and internal primers annealing with nucleotides 2716-2741 and 3250-3227 for the *pol* region were used (11).

PCR standardized conditions were followed including 6  $\mu$ L of reverse transcriptase (RT) or previous PCR product. 2.5 and 2 mM MgCl<sub>2</sub> for the *gag* and *pol* regions, respectively, 0.2 mM for each dNTP (Amersham Pharmacia, Madrid, Spain), 0.5  $\mu$ M for each primer, and 2 U Taq polymerase (Promega) in a 50- $\mu$ L final volume. [beta]-actin gen amplification was performed to confirm the presence of DNA in the extraction from spermatozoa suspension. Results were read after a 2% agarose gel electrophoresis after ethidium bromide staining (10). In all the samples, a consistent result (either positive or negative) was obtained. The assay failure rate was zero.

The quantitative one-round PCR technique was performed according to the method of Roche Diagnostic Systems (Amplicor; Roche, Basel, Switzerland) and as described in the work by Marina and coworkers (6). Briefly, RNA extraction was performed according to the Amplicor Specimen Preparation Kit (Roche, Barcelona, Spain).

To check for HIV RNA load, reverse transcription and amplification using SK145 and SKCC1B were carried out (amplifying a limited region of the HIV-1 *gag* gene, which translates the viral proteins p18, p24, and p55). The PCR-amplified product was detected and quantified through hybridization with the use of a specific biotin-labeling probe. An ELISA was used for subsequent detection.

To check for HCV RNA load, semen-washed samples were tested using a reverse transcription assay with a commercial kit for amplification and detection of HCV RNA (Amplicor HCV Amplification Kit and Detection Kit; Roche).

## RESULTS

Thirty-four seropositive men studied were infected by HIV by parenteral use of drugs ( $n = 17$ , 50%); sexual transmission, ( $n = 8$ , 23.5%); blood or blood product transfusion ( $n = 7$ , 20.5%), and unknown causes ( $n = 2$ , 5.8%). Twenty (58.8%) had undetectable levels of HIV RNA in blood before sperm wash; HIV RNA was detected in 14 patients with an average of 73,523 copies of HIV RNA/mL (range, 52-550,000). Twenty-one (61.7%) of the HIV-seropositive patients were co-infected with HCV. In four samples out of 41 (9.7%), sperm wash was not performed because the total amount of progressive motile sperm in the ejaculate was below 1 million. In two of these patients, a second collection on a different day provided enough sperm, while in last patient, after collection of the semen sample on two different occasions, the number of progressive motile sperm never reached 1 million and subsequently the treatment could not be performed.

As described in Table 1, five out of 41 samples (fraction of motile spermatozoa obtained after washing) were positive for HIV (12.2%) and five out of 21 were positive for HCV (23.8%) after the wash. Those samples were then confirmed with Amplicor Monitor RT-PCR (one-round PCR), and none were positive. Thus, the presence of HIV and HCV was lower than 200 IU/mL and 600 IU/mL for HIV and HCV, respectively.

Patients	Blood		Semen washes	Positives after wash (nested PCR)			Positives after wash (one-round PCR)	
	HIV+	HCV+		HIV		HCV	HIV	HCV
				DNA	RNA	RNA		
$n = 34$	34	22	41	5	2	5	None	None

five: HCV = hepatitis C virus; HIV = human immunodeficiency virus.  
Meseguer. HIV and HCV detection in washed sperm. Fertil Steril 2002.

TABLE 1 Summary of results of positive sperm washes and polymerase chain reaction (PCR) results.

[\[Help with image viewing\]](#)

## DISCUSSION

In this study, we have demonstrated that samples that were considered as negative by use of commercial methods for HIV/HCV detection are not absolutely free of virus, since nested PCR results were positive. Of the washed sperm analyzed, 12.5% were positive for HIV-1, and 23.8% for HCV.

This value is considerably higher than what has been reported by other groups such as Marina and coworkers for HIV-1, in which 5% of the samples tested positive after the treatment (7). This could surely be caused by the higher sensitivity of nested PCR, although the number of washes performed in our study is still low.

Moreover, washed spermatozoa cocultures with mononuclear blood cells were also negative even for positive samples with nested PCR (data not shown), suggesting that this is another method with insufficient sensitivity (12).

Nevertheless, we are conscious that no infection has been reported by using the one-round PCR in more than 500 AI cycles that have been reported on up to this time (13), and this could give sufficient reason to keep this system working.

But as Marina states in his paper, "...it is possible that a few spermatozoa contained HIV-1 and were not detected with the technique used." Thus, the risk of contamination exists. In our opinion, all guarantees should be given to the patients, providing more sensitive techniques if they are available. We will never recommend the use of a nested positive sample, even though the quantitative methods may give a negative result.

In addition, four points should be considered in the management of these patients, including the substitution of AI by IVF:

First, the use of frozen sperm allows the initiation of the stimulation cycle when sperm wash is negative, avoiding any cycle cancellation and permitting confirmation in cases of dubious results. Second, one negative wash can be employed in different cycles if pregnancies are not attained in the first attempt. This is in contrast to AIs, where the whole sample should be used at one time to inseminate a minimum number of motile sperm. Third, maternal exposure to potentially infecting sperm is reduced to the minimum (only one sperm will reach mother's uterus, as an embryo). Fourth, pregnancy rates per cycle are much higher in IVF than with AI, hence reducing the psychological stress in this per se stressed population.

## References

1. Bellver J, Meseguer M, Garrido N, Ruiz A. Virus de la inmunodeficiencia humana y reproducción. In: Remohí J, Simón C, Pellicer A, eds. Reproducción. Madrid: Humana McGraw-Hill, 2002. [\[Context Link\]](#)
2. Semprini A. Reproductive counselling for HIV-discordant couples. *Lancet* 1997;349:1401-2. [Ovid Full Text](#) [Bibliographic Links](#) [\[Context Link\]](#)
3. Caldwell SH, Sue M, Bowden JH, Dickson RC, Driscoll CJ, Yeaton P, et al. Hepatitis C virus in body fluids after liver transplantation. *Liver Transpl Surg* 1996;2:124-9. [Bibliographic Links](#) [\[Context Link\]](#)
4. Levy R, Tardy JC, Bourlet T, Cordonier HF, Mion J, Lornage J, et al. Transmission risk of hepatitis C virus in assisted reproduction techniques. *Hum Reprod* 2000;15:810-6. [Bibliographic Links](#) [\[Context Link\]](#)
5. Liou TC, Chang TT, Young KC, Lin XZ, Lin CY, Wu HL. Detection of HCV RNA in saliva, urine, seminal fluid ascites. *J Med Virol* 1992;37:197-202. [Bibliographic Links](#) [\[Context Link\]](#)
6. Semprini AE, Levi-Setti P, Bozzo M, Ravizza M, Taglioretti A, Sulpizio P, et al. Insemination of HIV-negative women with processed semen of HIV-positive partners. *Lancet* 1992;340:1317-9. [Bibliographic Links](#) [\[Context Link\]](#)
7. Marina S, Marina F, Alcolea R, Exposito R, Huget J, Nadal J, et al. Human immunodeficiency virus type 1-serodiscordant couples can bear healthy children after undergoing intrauterine insemination. *Fertil Steril* 1998;70:35-9. [Ovid Full Text](#) [Bibliographic Links](#) [\[Context Link\]](#)

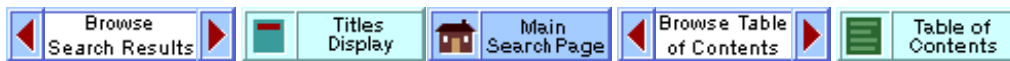
8. Hanabusa H, Kuji N, Kato S, Tagami H, Kaneko S, Tanaka H, et al. An evaluation of semen processing methods for eliminating HIV-1. *AIDS* 2000;14:1611-6. [Ovid Full Text](#) [Bibliographic Links](#) [\[Context Link\]](#)
9. Pasquier C, Daudin M, Righi L, Thauvin L, Izopet J, Bujan L. Sperm washing and virus nucleic acid detection to reduce HIV and HCV transmission in serodiscordant couples (male partner infected wishing children). *Fertil Steril* 2000;74:S38. [\[Context Link\]](#)
10. Meseguer M, Aplin JD, Caballero-Campo P, O'Connor Enrique, Martín JL, Remohí JL, et al. Human endometrial MUC1 is up-regulated by progesterone and down-regulated in vitro by the human blastocyst. *Biol Reprod* 2001;64:590-601. [Bibliographic Links](#) [\[Context Link\]](#)
11. Zazzi M, Romano L, Catucci M, De Milito A, Almi P, Gonnelli A, et al. Low human immunodeficiency virus type 1 (HIV-1) DNA burden as a major cause for failure to detect HIV-1 DNA in clinical specimens by PCR. *J Clin Microbiol* 1995;33:205-8. [Bibliographic Links](#) [\[Context Link\]](#)
12. Merigan TC. Use of PCR to measure HIV viral changes in drug-resistant genes in genital fluids. *J Reprod Immunol* 1998;41:177-85. [Bibliographic Links](#) [\[Context Link\]](#)
13. Marina S, Marina F, Exposito R, Alcolea R, Perez N, Bermejo M, et al. Sperm washed in infected HIV-1 men for assisted reproduction. In Program and Abstracts of the X Meeting of the Asociación Española de Andrología (ASESA), Alicante, Spain, P4. [\[Context Link\]](#)

Key Words: HIV; HCV; semen wash; nested PCR; serodiscordant

---

*Accession Number: 00003748-200212000-00011*

---



Copyright (c) 2000-2004 [Ovid Technologies, Inc.](#)  
Version: rel9.1.0, SourceID 1.9087.1.155