



SPAR · SPECIAL PROGRAM OF ASSISTED REPRODUCTION

A Program of the Bedford Research Foundation Clinical Laboratory

Massachusetts 501 (c)(3), not for profit organization

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Understanding the Science

A guide to the differences between HIV in blood and HIV in semen, the HIV semen test, “sperm washing,” and how SPAR developed the most stringent safety standards for semen testing in the world.

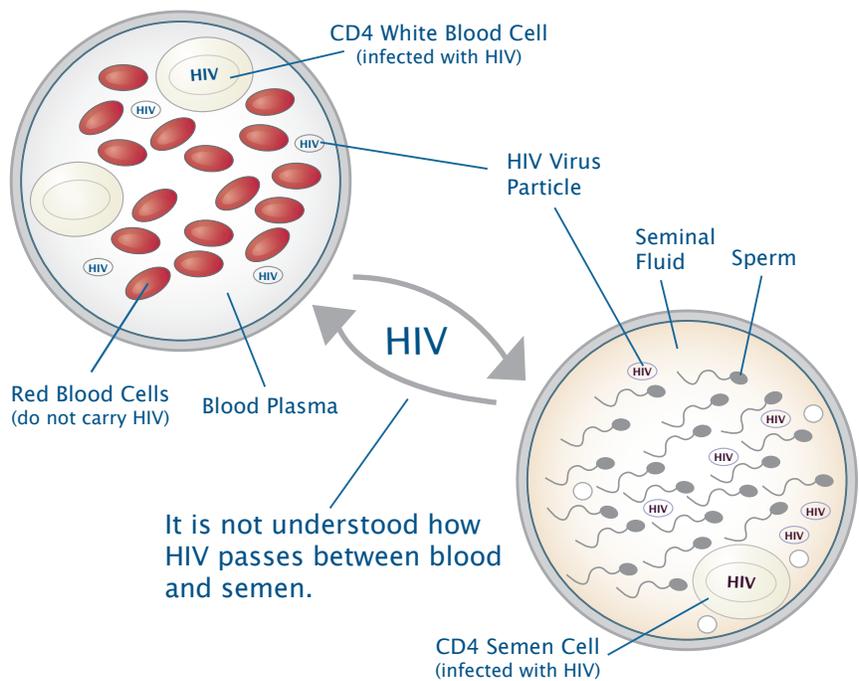
HIV in Blood VS HIV in Semen

Research has shown that an HIV blood test is *not* a reliable predictor of viral burden in semen. These studies have revealed that semen producing organs are a separate compartment of HIV infection, and that semen viral burden is different from blood viral burden, even in men on therapy. For this reason, Bedford scientists have structured SPAR around semen testing and using sperm only from specimens that test *undetectable* for HIV.

Research suggests that virus replication is compartmentalized between blood and semen.

An undetectable viral burden in blood may not indicate an undetectable burden in semen.

Treating HIV in blood may not treat HIV in semen.



Data compiled by BRF scientists in 2006 revealed that 24% of the semen specimens from 262 men entering SPAR were positive for HIV, even though the men had an undetectable burden of viral RNA (HIV particles) in blood. All men eventually produced at least two specimens with undetectable virus.

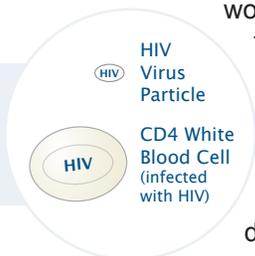
This important finding, that HIV is intermittently present in semen specimens, is what makes testing each specimen for virus so important to prevent virus transmission.

HIV Biology

Human Immunodeficiency Virus (HIV) infects cells by first binding to specific receptors on the cell surface, then entering the cell. HIV uses two receptors to gain entry. One receptor is termed CD4 and is on the surface of cells belonging to the immune system.

Once inside the cell, HIV engineers a copy of its genetic information to become an integral part of the cell's genes. It does not kill the cell it has infected, but it takes advantage of the cell's machinery to make new virus particles that are released from the surface of the cell. The new virus then attaches to a new, uninfected (CD4) cell, to repeat the process.

Therefore, HIV infection has two forms in the body: *free virus particles*, and *virus-infected cells*.



Not all virus-infected cells produce virus particles all the time. Some infected cells do not produce new virus particles unless they are stimulated to respond to another type of infection, such as the flu, or a bacterial infection. Once stimulated, they begin to produce new HIV particles, and then die. Because CD4-cells are a key player in immune responses to all types of infections, the relentless loss that results from HIV infection gradually erodes the immune system, which leads to AIDS.

Most of the HIV-infected cells are in lymph nodes, a few circulate in the blood stream, and others are scattered throughout the body. Importantly, even after 25 years of research, the organ source of semen HIV is unknown.

Semen Biology

Semen is a complex and unusual body fluid. It does not exist anywhere within the body, it is created by ejaculation of cells and fluids from three tissues (epididymis, seminal vesicles and prostate) carried by two duct systems (vas deferens and urethra). Each semen specimen is unique.

The testis produces millions of sperm each day which take about 6 weeks to mature from large round immature cells (spermatogonia) to stream-lined cells with a tail that moves. The sperm are produced inside tubes within the testis which protects them from the outside world. Once they gain tails, they leave the testis and enter the epididymis. Their transit through the epididymis takes another few weeks, and they are stored near the vas deferens, the tube that connects the testis/epididymis with the urethra in the penis.

At ejaculation, muscles in the epididymis, vas deferens, seminal vesicles and prostate all contract to force sperm and fluids out the opening of the urethra. The

average specimen is about half a teaspoon in volume and contains approximately 100 million sperm and 2 million non-sperm cells. The non-sperm cells are either immature sperm that left the testis prematurely, and/or cells that belong to the immune system, including CD4-cells that come from the epididymis, the prostate or seminal vesicles, and that could be infected with HIV.

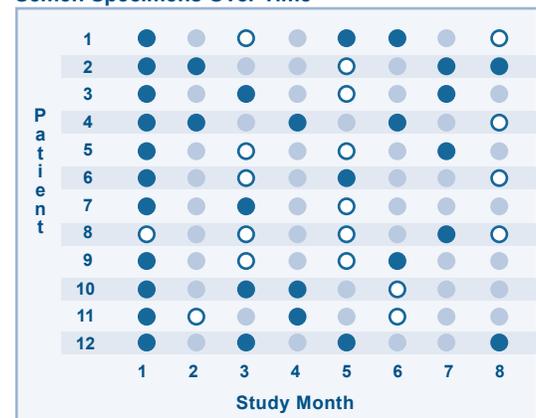
HIV therapy

HIV is the first viral epidemic to be treated by drugs instead of vaccines. To date there are about two dozen antiretrovirals, plus several drug combinations. The drugs either inhibit virus entry into cells, or inhibit some part of the HIV life cycle so the production of new virus particles is halted. Effective antiretroviral therapy reduces the number of free virus particles circulating in blood to zero, but it does not eliminate HIV infected cells in circulation, and it may not eliminate HIV from semen specimens.

Early in the development of SPAR, 12 men agreed to provide semen specimens over an 8-month interval. Each specimen was tested for HIV. Patients 1 and 2 were not on anti-viral therapy. Patients 3 and 4 were beginning anti-viral therapy. Patients 5 through 12 were on stable anti-viral therapy with an undetectable (fewer than 400 copies of HIV RNA per cc) viral load in blood.

- HIV Detected
- HIV Not Detected
- No specimen that month

**Sample of Study Data
Semen Specimens Over Time**



The Bedford Research Foundation's clinical laboratory is a not for profit, Massachusetts public charity. The laboratory operates on the principle that revenues and information from existing laboratory tests should be used to support research and the development of new tests.

PCR HIV Test for Semen

Bedford Research Foundation uses the most precise form of HIV testing available for semen.

The specimen is divided into three portions - one portion is RT-PCR tested for HIV, a second portion is immunostained for cells of the immune system. Sperm are recovered from the third portion, washed, and cryopreserved in liquid nitrogen (back cover).

The PCR HIV DNA assay took several years to develop. It is a molecular biology test similar to the one used for quantitation of blood virus, but more sensitive because of the small size of the semen specimen. It is designed to detect both free virus and virus infected cells. (The assays have not been FDA approved.)

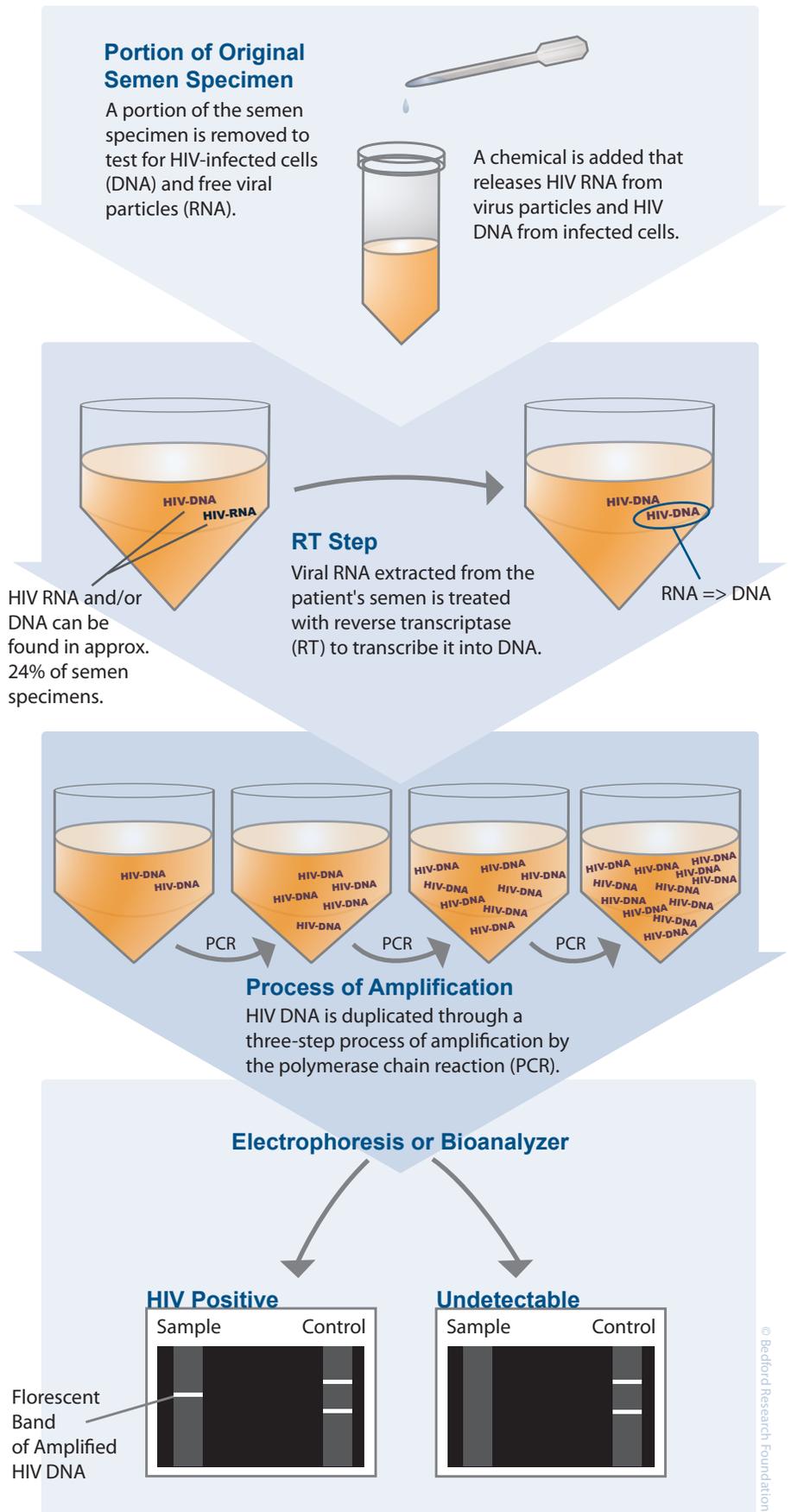
The first step is to convert all the HIV-RNA to HIV-DNA using RT¹. Second, the DNA in the sample must be amplified by PCR¹.

The process of amplification allows a small number of copies of HIV-DNA to be detected by electrophoresis through agarose gels or an Agilent Bioanalyzer. A normal cellular gene is amplified in the same test to serve as an internal control.

¹RT-PCR stands for Reverse Transcription - Polymerase Chain Reaction. Reverse Transcription (RT) uses a specific enzyme to convert RNA to DNA.

The Polymerase Chain Reaction (PCR) amplifies highly specific DNAs by employing repeat cycles of heating and cooling to the assay tube that contains HIV-specific reagents.

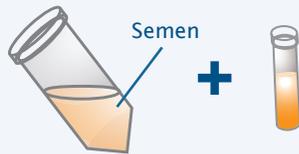
Although commonly considered one of the most accurate and specific HIV tests available - PCR testing is not typically used for standard HIV blood tests because the time consuming process is expensive.



Sperm Washing

Sperm washing is a standard procedure used in infertility treatments. Sperm are separated from seminal fluids using a centrifuge.

Because HIV has been found in the seminal fluid and in semen cells, *but not sperm*, it is believed that separating the sperm from the seminal fluid will lower the risk of infection.



A solution is added to the seminal fluid that is denser than the seminal fluid and immune cells, but less dense than sperm.



The centrifuge spins at a high speed, separating the sperm from the seminal fluid.



After the specimens have been spun in the centrifuge, the sperm are concentrated in the bottom of the test tube. The upper layers of seminal fluid and non-sperm cells are discarded. The washed sperm are re-suspended in a new solution for cryo-preservation.

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Semen Analysis for Infertility & Complicating Factors

In addition to HIV viral testing and sperm washing, each semen specimen undergoes a standard semen analysis to evaluate male fertility and/or detect problems in male organs.

Semen Analysis for Infertility

This basic evaluation of the semen specimen includes:

➤ Sperm Count

The sperm count (concentration) is a measure of the total number of sperm (spermatozoa) present. It is recorded in millions of sperm per milliliter of semen. A normal count is greater than (\geq) 20 million per ml. A lower than normal concentration (sperm count) can be used in many infertility procedures.

➤ Motility

This measures what percentage of sperm are moving and how well they are moving. Normal semen will have at least 50% motile sperm.

➤ Morphology

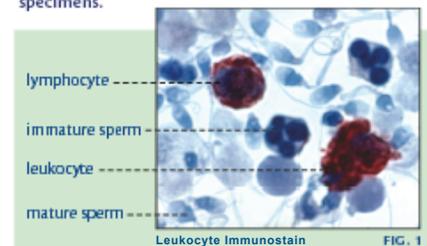
Sperm Morphology is the *form* and *structure* of a single sperm. Sperm are evaluated by both WHO (World Health Organization), 1994 criteria (Norm forms WHO) and Kruger (Fertil Steril 49:112, 1988) strict criteria (Norm Forms Kruger). Normal Forms Kruger is ≥ 14 , Normal Forms WHO is ≥ 60 .

Leukocyte Immunostain

If a semen specimen has greater than one million/ml non-sperm cells, an immunostain will be performed. The immunostain will color immune cells (leukocytes) red-brown, thus distinguishing them from immature sperm. Since immune cells may carry the virus, a high count may indicate a higher risk of virus transmission through the sample. The patient may need to see a urologist to correct the

problem of high immune cells in semen.

Bedford Research Foundation is the only clinical lab in the United States licensed to immunostain semen specimens.



Leukocyte Immunostain FIG. 1

Cryopreservation

Cryopreservation is the process of freezing sperm in liquid nitrogen so that they remain alive and may be shipped to a cooperating fertility center for use in conception.

Bedford scientists will only store cryopreserved sperm from semen specimens that have an undetectable viral burden using the PCR test.

Specimens will be stored until you require them.

LIN
Nitrogen
Refrigerated
Liquid

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