

*Infectious HIV in Breastmilk:
Fact or Fantasy?*

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“Fear of HIV transmission is the biggest threat to breastfeeding in the Third World, where a large fraction of women are HIV-positive”

Objective

To weigh the evidence for HIV existing as infectious particles in breastmilk.

Detecting HIV in Breastmilk

Basic Test Definitions

Antibody Evidence

Antigen Evidence

DNA PCR Evidence

RNA PCR Evidence

Co-Culture Evidence

Gold Standard

But, I was told...

Basic Test Definitions

| | |
|---|---|
| Specificity | Fraction of uninfected people who test negative. |
| Sensitivity | Fraction of infected people who test positive. |
| Reproducibility | Tests produce same results on same specimen when run by different labs, different people, etc. No substitute for Specificity and Sensitivity! |
| “Gold Standard” | Unambiguous evidence for presence of a pathogen. Used to ‘test the tests’. |
| The ‘Rare Condition’ Testing Dilemma | If a test is 99% specific (1/100 people test false positive) and is used in a population where 1/1000 people are actually infected, 9 out of 10 positive results will be false! |

Antibody Evidence

- **Antibody:** a protein produced by the body to react with proteins from the pathogen (antigens).
- **Detection:** placing serum in contact with HIV antigens, e.g. in the ELISA or Western Blot tests.
- Antibodies are passed from mother to child via breastmilk
- Antibodies are evidence for *past* exposure to a virus, not active infection
- Antibodies can cross-react with other antigens from other pathogens or other conditions (e.g. auto-immunity)

Flies in the Ointment?

- [Burke, 1988] calculated high accuracy by using an artificially high number of tests (7). But using the normal ELISA+ELISA+WB:
“approximately 1% of all initial screening ELISAs were reactive, 50% of repeat ELISAs were reactive, and 30% to 40% of first Western blot assays were reactive and diagnostic.”
- [Kion, 1991] made lab mice produce antibodies to HIV:
“Alloimmune mice...were shown to make antibodies against gp120 and p24 of human immunodeficiency virus (HIV), and mice of [two] autoimmune strains...made antibodies against gp120. This is surprising because the mice were not exposed to HIV.” Also see [Strandstrom, 1990] with dogs.
- Not everybody with AIDS has HIV antibodies. [Gallo, 1985] found that 88% tested positive and [Weiss, 1985] found 82%.
- [Minkoff, 1998] notes that “15%-20% of [antibody] tests from low-risk patients will be indeterminate and remain so...over many months.”

HIV Antibodies & Breastmilk

- Antibodies in breastmilk and in babies may come from the mother, and cannot be construed as a sign of an active infection.
- The assumption that at 9, 12, 15 or 18 months antibodies in children reflect an active infection is widely used, but highly questionable.
- Just because many (not all) people with AIDS have somewhat similar antibodies does not mean that the antibodies are associated with a virus, and that this virus is the cause of AIDS!
- Simple antibody tests (ELISA) are validated with more complex antibody tests (WB), a sophisticated form of circular reasoning.

Conclusion: Antibodies *cannot* be used to detect infectious HIV in breastmilk

Antigen Evidence

Antigen: a protein that is believed to come from HIV

Detection: by reaction of serum with antibodies to (usually) p24

- Antigens can cross-react with other antibodies
- Antigen tests detect proteins, not necessarily infectious virus particles
- Should be taken more seriously than antibody tests, but is actually given less weight than antibody tests

Prone to Problems?

- [Blanche, 1994] notes that p24 is rare in HIV+ mothers:
“At the time of delivery, HIV-1 p24 antigen was detected in serum from 16 of 108 [HIV+] women (15%)”
- [Fischl, 1990] found only half of HIV(antibody)+ people had p24:
“205 subjects (of 406 tested (50%)) had detectable serum levels of HIV antigen before treatment”
- [Faulk, 1991] found HIV antigens in *uninfected* human placentas:
“Cryostat sections of human normal term placentae were...examined for HIV protein antigens gp120, p17, p24, and gp41...Antigens gp120 and p17 were identified in normal chorionic villi in vimentin-positive fibroblast-like cells and in endothelium, respectively. Antigen p24 was localized to HLA-DR positive cells that morphologically resembled macrophages in areas of villitis.”
- [Urano, 1994] note that these tests have low *Specificity* and *Sensitivity*:
“p24 antigen was detected in 6 [of 32] patients of group P [positive] and 2 [of 27] patients of group N [HIV-negative]. ”

What do Antigens Tell us?

- Antigen tests cannot distinguish infectious from uninfected particles nor true reactions from false reactions.
- Antigen tests appear to be very misleading

Conclusion: Antigen tests cannot be used to prove the presence of infectious HIV in breastmilk.

DNA PCR Evidence

HIV DNA: “Reverse Transcribed” copy of HIV genome inserted into human DNA

Detection: Using Polymerase Chain Reaction (PCR) or similar techniques

Purpose: To count the number of infected cells in a sample

- Known as the ‘viral load’ test
- A small fraction of the HIV genome is used as a primer
- Assumes that the primer is ‘conserved’ and not found in other viruses, including Human Endogenous Retroviruses (HERVs)
- PCR is an exponential reaction, so errors multiply exponentially
- Measures DNA, does not count virus particles, despite the name

RNA PCR Evidence

- HIV RNA:** Genetic material of HIV within the virus particle
- Viral Load:** Term used to describe numbers derived from PCR calculations.
- Detection:** Using Polymerase Chain Reaction (PCR) or similar techniques
- Purpose:** To count the number of virus particles in a sample
- Cannot distinguish between infectious and non-infectious particles
 - The vast majority of PCR measurements (e.g. 59,999 out of 60,000) appears to indicate non-infectious (non-co-culturable) RNA

Questioning PCR

- [Busch, 1992] found poor sensitivity, specificity and reproducibility:
"This proficiency study of PCR detection of HIV-1 DNA in serum identified a disturbingly high rate of nonspecific positivity with a widely employed gag primer pair system. In fact, the overall rate of positivity was not significantly different for serum specimens from seropositive patients and seronegative control donors (26% versus 18%)"
- [Mendoza, 1998] found a 5-20% rate of false positives among healthy, HIV-negative people.
- [Dunn, 2000] found this super-sensitive test to not be sensitive enough:
"Although DNA and RNA PCR and cell culture can detect very low concentrations of HIV-1, these assays yield a positive result in only 20-40% of vertically-infected infants who are tested shortly after birth"

Questioning PCR (cont'd)

- [Piatak, 1993] estimated that 59,999 out of 60,000 particles counted are not infectious:
“Circulating levels of plasma virus determined by QC-PCR also correlated with, but exceeded by an average of nearly 60,000-fold..., titers [amounts] of infectious HIV-1 determined by [co-culture].”
- [Rich, 1999] doesn't recommend PCR for diagnosis of infection:
“Plasma viral load tests for HIV-1 were neither developed nor evaluated for the diagnosis of HIV-1 infection; therefore their diagnostic specificity is not well delineated when applied to persons who are negative for HIV antibody. We report two cases of false-positive results...”
- Even the manufacturers are dubious [Roche, 1996]:
“The AMPLICOR HIV-1 MONITOR Test is an in vitro nucleic acid amplification test for the quantitation of Human Immunodeficiency Virus Type 1 (HIV-1) RNA in human plasma... [It] is not intended to be used as a screening test for HIV or as a diagnostic test to confirm the presence of HIV infection”

Using PCR on Breastmilk

- Viral load tests do not show that infectious HIV is present.
- Cells containing HIV DNA or Virions may be inactivated by breastmilk, saliva or the infant's gut...but still register positive.
- Highly sensitive. No guarantee that low levels of virus detectable by this incredibly sensitive test are biologically active enough to cause disease.
- Test not approved by FDA for diagnostic purposes.
- Tests not validated against actual virus particle counts (*Gold Standard*).
- *Lack of alternatives does not validate this test!*

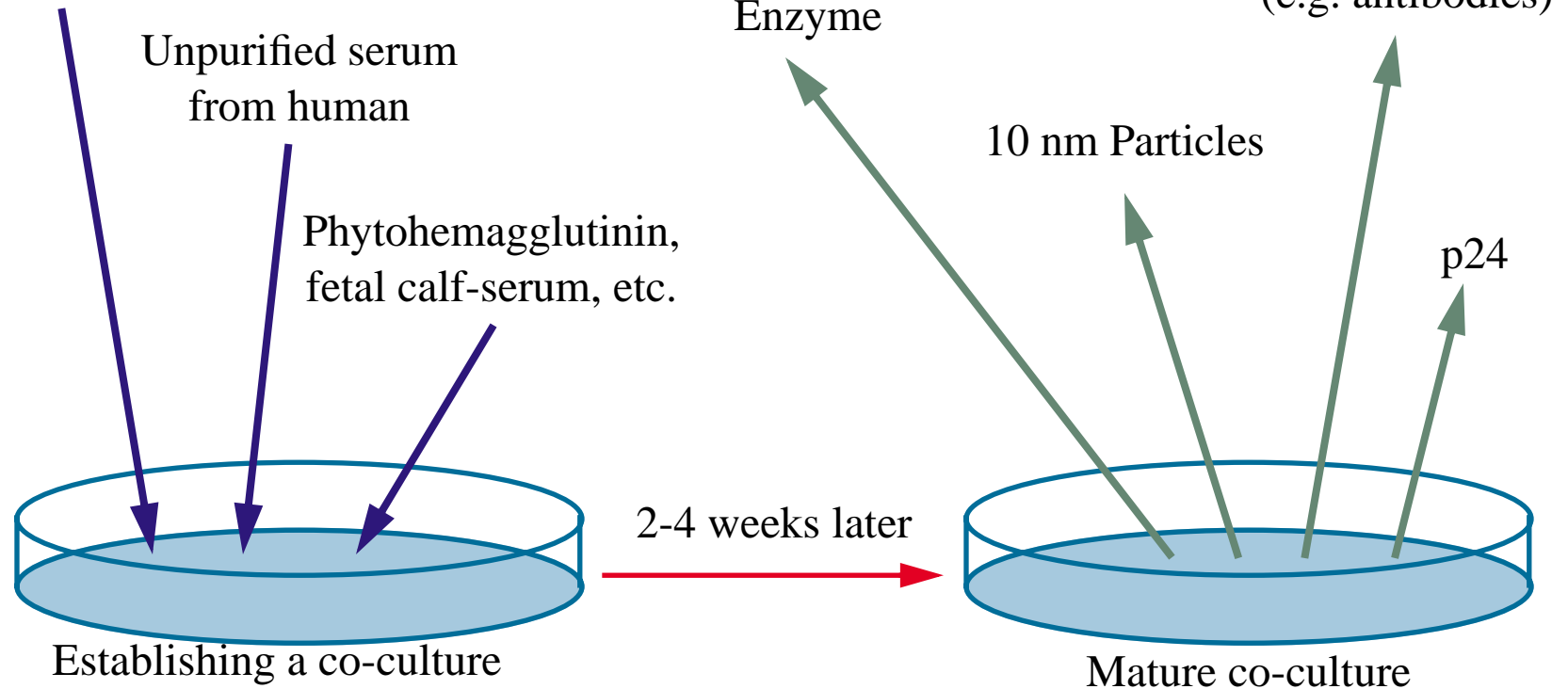
Conclusion: Detection of HIV DNA or RNA using PCR does not prove presence of active HIV.

Co-Culture Evidence

- Co-Culture:** Cell culture infected with a pathogen that cannot grow by itself.
- Setup:** Unpurified serum and stimulating chemicals added to a cell culture (often an immortal cancerous cell line).
- Detection:** Presence of non-specific phenomena: Reverse Transcriptase activity, p24 antigen, antibody-containing cells, particles of the expected size in the unpurified culture, etc.
- Often erroneously called 'culture' (minor error)
 - Often called 'isolation' (grievous error)
 - Believed to be the closest to a *Gold Standard* available in HIV research

How Co-Culture Works

Cell culture (e.g. cancerous lymphocytes - T Cells)



What's wrong with this picture?

Co-Culture Conundrums

- [Imagawa, 1989] document many with positive co-culture who stay negative:
“225 [co-]cultures of peripheral-blood lymphocytes from 133 seronegative men [at high risk of HIV infection] were performed, and HIV-1 was isolated [sic] in cultures from 31 men (23%). Of these men, four have seroconverted after being seronegative for 11 to 17 months after the initial isolation of the virus. The virus was not always isolated at every visit after the first successful isolation”
- [Michaelis, 1987] show co-culture not concordant with other tests:
“Virus was isolated [sic] from...approximately 50% of [39 HIV+] individuals and 1/3 also yielded infectious virus in their serum. Three serum samples contained infectious HIV without any virus being recovered from the individuals' PMCs [peripheral mononuclear cells]...not all seropositive individuals have virus recoverable from their PMCs...isolation from serum is not a common event”

What Co-Culture Tells Us

- Co-culture should be initiated with purified virus particles (but isn't).
- Co-culture should be tested by purification of larger amounts of virus than it was innoculated with (but isn't).
- Stimulation of cells can activate Human Endogenous Retroviruses (HERV), that can act like external retroviruses.

Conclusion: Co-culture cannot be considered valid unless it is compared against a *Gold Standard* or used more rigorously.

Gold Standard:

Purification & Isolation of HIV

- HIV has never been purified from any human serum, including breast-milk.
- HIV has never been purified from a co-culture. Claims that extracts of co-culture were 'pure' were proven wrong by [Gluschankof, 1997] and [Bess, 1997].
- Isolation of the virus is necessary to determine what the HIV RNA and proteins (antigens) are.
- Isolation of HIV requires purification
- HIV tests must be validated against the only possible *Gold Standard*, proof of the presence of HIV in (and only in) samples that test positive

Conclusion: No HIV tests have been properly validated

But I was told...

- Many papers have claimed ‘isolation’ of HIV in breastmilk and other substances, but none have actually separated viral particles from their surroundings!
- Decision of CDC and other agencies to recommend against breastfeeding by HIV-positive mothers was based on [Thiry, 1985] and [Ziegler, 1985]
- [Thiry, 1985] studied 3 healthy, HIV+ mothers and found markers for HIV in the breastmilk.
- [Ziegler, 1985] studied one HIV+ child of a mother given an HIV+ transfusion after birth. Neither mother nor child had AIDS. Father, brother and sister were HIV-negative. HIV status of mother before transfusion was unknown. No evidence that HIV was present in the breastmilk was provided.

Epidemiologic Evidence:

For the Prosecution

Postnatal HIV transmission

Excess of HIV transmission in breastfeeding mothers

For the Defence

Consideration of 'exclusive' breastfeeding

Balance of risks

Postnatal HIV transmission

- Mother transfused at or after birth
- Baby later found to be HIV-positive
- HIV status of mother, breastmilk and blood often not known
- No control group (i.e. non-breastfeeding mother in same circumstances)
- No reason to publish similar cases where HIV transmission does not occur.
- Very small number of such cases due to unique circumstances, e.g. [Ziegler, 1985], [Lepage, 1987], [Weinbreck, 1988], [Colebunders, 1988], [Stiehm, 1991]
- Even if false-positive HIV test results are extremely rare, they could easily explain such a small number of cases

Excess of HIV transmission

- Comparison of fraction of HIV+ babies of mothers known to be HIV+ at delivery in breastfeeding versus formula-feeding groups
- Excess in breastfeeding groups *assumed* to be due to transmission via breastmilk, and not due to other differences between the groups.
- Some studies show evidence of ‘randomization’ errors (e.g. [Nduati, 2000]) that may indicate bias in the studies.
- [Dunn, 1992] summarized 6 previous studies (some unpublished) and estimated an excess risk of 14%. Other analyses of the same numbers produced estimates of 9, 11, 16 or 18%, due to the small number of HIV+, nursing mothers studied and large differences between studies.
- No consistent definition of what ‘breastfeeding’ is, specifically an extensive period (e.g. 6-12 months) of exclusive breastfeeding versus mixed-feeding.

Exclusive breastfeeding

- [Coutsoudis, 2001] found an insignificant difference between HIV transmission among exclusively breastfed and exclusively formula fed infants.
- Mixed fed infants had a higher incidence of transmission (26% versus 19%)
- There is no good explanation for these findings
- This work calls all previous epidemiologic studies into question.

Balance of risks

Question: Is a breastfed, HIV+ baby necessarily worse off than a formula fed, HIV-negative baby?

- [Nduati, 2000] found no difference in 2-year survival between HIV+ and HIV- babies, so invented the term “HIV-free survival” to equate “HIV+” with “soon to be dead”.
- [Ryder, 1991] found that exclusively breastfed babies were healthier than formula fed babies of the same HIV status.
- [Dunn, 1992] showed that breastfeeding won't change the HIV status of the vast majority of babies.
- The mean time from HIV seroconversion to AIDS is over 10 years [Munoz, 1997]. By then, many formula-fed babies will be dead.
- There is a correlation between ill health of the mother and her baby [Kind, 1992].

Summary

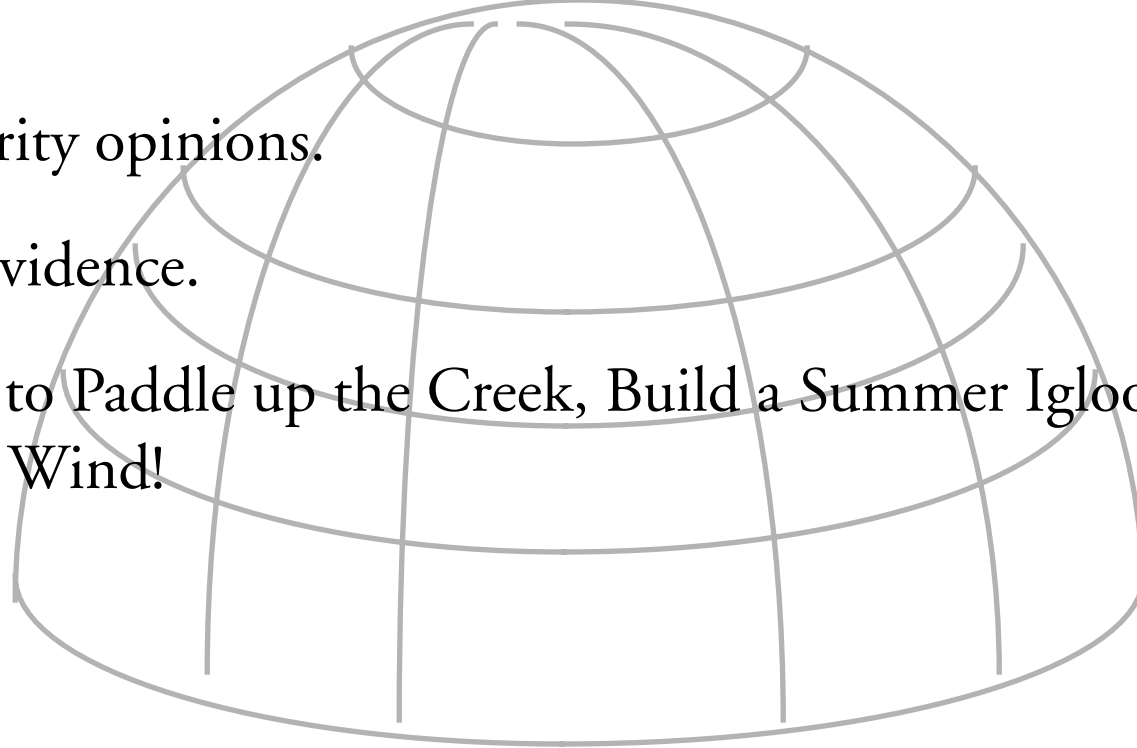
- There is no test that can directly detect HIV in breastmilk.
- All indirect tests are subject to false results, particularly in third world countries.
- Epidemiologic studies rely on fallible test results, and usually consider only HIV status and not health
- Unquestioning acceptance of current dogmas about HIV and AIDS is one of the biggest threats to breastfeeding
- Discouraging breastfeeding by HIV+ mothers will have known major negative health effects
- Labelling mothers HIV+ will lead to neglect of them and their babies [Grinstead, 2001]

Your Challenge

- Accept the current dogmas about HIV, breastfeeding and AIDS, and watch breastfeeding retreat in the Third World

or

- Ignore majority opinions.
- Weigh the Evidence.
- Be prepared to Paddle up the Creek, Build a Summer Igloo, Sail into the Wind!



Resources

- <http://www.virusmyth.com> - many links
- <http://aras.ab.ca> - referenced quotes on testing, surrogate markers and negative effects of AIDS drugs. Plus links.
- Duesberg PH. *Inventing the AIDS Virus*. Regnery. 1996.
- Maggiore C. *What if everything you thought you knew about AIDS was wrong?* American Foundation for AIDS Alternatives. 2000.
[Available from our society or from aliveandwell.org]
- Package of papers by the 'Perth Group', available at no charge from Dr. Valendar Turner (Email: vturner@cyllene.uwa.edu.au)

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