

[BACK](#)

THE HIV GENOME

"...analysis of the proteins of the virus demands mass production and purification...I repeat, we did not purify"

Luc Montagnier, Pasteur Institute, July 18th 1997

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INTRODUCTION

It is a recognised fact that in clinical practice the ultimate proof of ownership of organs, tissues, blood or other biological material relies entirely on documenting their removal from the body of a unique member of the species *Homo sapiens*. As every doctor knows medical practice abounds with virtually forensic precautions for designating and preserving the identity of such specimens as they pass from patient to laboratory for processing. The same rigour is demanded by courts of law when it comes to admissibility of evidence in regard to blood alcohol readings or DNA analysis. Likewise, proof that particular biochemical entities are viral must follow the same standard of proof, that is, the principle we may call virological *habeas corpus*.

If a fragment of RNA is the genome of a retrovirus then proof must exist that the fragment originated from a retroviral particle. Since it is not possible to obtain the RNA of a single particle the only alternative is to obtain it from material which contains nothing else (or at least nothing containing nucleic acids) but particles having the morphological characteristics of retroviruses. That is, the retrovirus particles must be isolated/purified. The method of choice to achieve purification is banding in density gradients. In this procedure an aliquot of cell culture supernatant is placed at the top of a column of sucrose solution contained in a centrifuge tube.

The solution is prepared such that its density increases gradually from top to bottom. The tube is spun at very high speeds for many hours and if the supernatant contains retrovirus particles they will aggregate (band) in that portion of gradient where the density reaches 1.16 g/ml.^{1,2}

THE ORIGIN OF THE HIV GENOME

In 1983/84 two groups, one from the Pasteur Institute in Paris lead by Luc Montagnier and the other from the NIH lead by Robert Gallo, established highly stimulated cultures containing umbilical cord lymphocytes or transformed cells including leukaemic cell lines to which they added tissues from patients with AIDS or at risk of AIDS. They also added many other agents including irradiated cells of healthy blood donors as well as PHA, an agent which by itself is sufficient to cause the appearance of novel poly(A)-RNA (adenine rich RNA) in normal T lymphocytes.³ The supernatant from these cultures was spun in sucrose density gradients to purify the retrovirus which they postulated was present in AIDS patients and thus in the cultures. Both groups claimed to have achieved purification of a retrovirus which later became known as HIV.

However, for some unknown reason neither group published electron micrographs (EM) to prove that the 1.16 g/ml band contained nothing else but retroviral particles, that is, purified HIV.⁴⁻⁶ In July 1997, in an interview given to the French journalist Djamel Tahi, Montagnier was asked why he and his colleagues did not publish such EM to prove that the band represented purified virus, as they had claimed. He replied the reason was because even after a "Roman effort" examining EM of their "purified" virus they could not find any particles with the "morphology typical of retroviruses".⁷ Asked if Gallo purified HIV Montagnier replied: "I do not believe so".

By 1997 some of the best known HIV experts acknowledged that "HIV" "used for biochemical and serological analyses or as an immunogen is frequently prepared by centrifugation through sucrose gradients", and that in none of the studies "has the purity of the virus preparation been verified".⁸ In that year two studies were published, one by a USA team, principal author Julian Bess, and the other by a Franco-German group, principal author Pablo Gluschankof. These papers were the first ever published with EM of "purified HIV". The authors of both studies claimed that their "purified" material contained some particles with the appearances of retroviruses and in fact that they were HIV particles. But they admitted their material consisted predominantly of particles which were not retroviruses but "budding membrane particles frequently called microvesicles" or "mock virus". Indeed the caption to the Gluschankof *et al* electron micrograph reads, "Purified vesicles from infected H9 cells (a) and activated PBMC (b) supernatants" — not purified HIV. In further experiments the supernatant from non-infected cultures was also banded in sucrose gradients. Both groups claimed that the banded material from these cultures contained only microvesicles, "mock virus" particles, but no HIV particles. Both the "HIV" particles and the mock-virus particles possessed membranes. In the USA study the "HIV-1 particles" were differentiated from the microvesicles "by the electron dense cores" whereas in the other study the "HIV" particles were "identified by the relatively homogenous diameter of about 110nm, the dense cone-shaped core, and the "lateral bodies"". However, in the arrowed particles, which are said to be HIV, it is difficult if not impossible to locate any in which there are cone-shaped cores or bilateral, "lateral bodies". In fact no particle in any study has the principle morphological characteristics of a retrovirus, that is, "a diameter of 100-120 nm...and are studded with projections (spikes, knobs)".^{9,10} In the Franco-German study the average "HIV" particle diameter is 136 nM and no particle had a diameter less than 120 nM. In the USA study the corresponding dimensions are 236 nM and 160 nM.

Since (a) as Montagnier acknowledges, to characterise the HIV proteins and genome particle purification is necessary; (b) neither Montagnier's nor Gallo's team nor anybody else since has obtained purified retroviral particles then it is clearly not possible to characterise the HIV proteins and genome.

It goes without saying that if, in the "purified" virus there were no particles with the morphological characteristics of retroviruses, then any proteins present cannot be "HIV" proteins or those of any other retrovirus. Nonetheless, both groups claimed that some but not all of the proteins which banded in the 1.16 g/ml band, that is, those present in the "purified" virus, were HIV proteins. The only proof offered for this claim was that the proteins reacted with antibodies present in AIDS patient sera. This means that from a protein-antibody reaction they claimed to have proven the "HIV" origin of both the proteins and the antibodies.^{4-6,11,12} However, from a protein-antibody reaction it is not possible to prove the origin of even one reactant. Even if we assume the proteins are HIV proteins their reaction with antibodies present in patient sera does not prove the antibodies are directed against HIV. This is because: (a) antibodies including monoclonal antibodies¹³⁻¹⁵ react non-specifically,¹⁴⁻²³ (b) sera of both AIDS patients and those at risk have antibodies directed against a plethora of self and non-self antigens.²⁴

If in the 1.16 g/ml band, the "purified" virus, there were no particles with the morphological characteristics of retroviruses any RNA present cannot be the genome of HIV or any other retrovirus. Again, if the 1.16 g/ml band was indeed "purified" virus then the only nucleic acid present in the band should be RNA and not DNA because retroviruses are RNA viruses by definition. It also follows that all of the RNA present should be retroviral RNA.

Neither group stated if the "purified" virus contained DNA or what amounts and types of RNA were present. Instead, from the "purified" virus they selected a number of poly(A)-RNA fragments and claimed this RNA was the HIV genome. This claim was based on work done a decade earlier, including by Gallo and his colleagues, who showed that retroviral RNAs contain poly(A) regions and hypothesised "therefore that poly A might be a diagnostic property of tumour viruses [retroviruses]".²⁵ However, at that time evidence also existed (of which Gallo was aware), that poly(A)-RNA is not specific to retroviruses. Indeed, "poly-A sequences were found in both messenger RNA (mRNA) and their nuclear precursors...poly(A) sequences provided the basis for a long-sought route for mRNA purification...and generating the cDNAs and the probes derived from them on which so many studies of gene expression continue to depend". In fact poly(A)-RNA is found not only in all cells and retroviruses but also in other viruses.²⁶ Significantly, both Montagnier's and Gallo's group omitted to use controls. That is, banded material originating from cultures which, with one exception (instead of containing material derived from AIDS patients they should have material derived from non-AIDS patients), were identical to the cultures from which the "purified" virus was obtained. As mentioned, Bess and his colleagues obtained banded material from non-"infected" cultures and showed that the "mock" virus contained RNA, including mRNA, that is poly(A)-RNA. The poly(A)-RNA which Montagnier and Gallo's group obtained, the "HIV" RNA, was reverse transcribed and then the cDNA cloned. The clones thus obtained were sequenced and used as hybridisation probes and PCR primers.^{27,28}

The Pasteur researchers wrote: "The complete 9193-nucleotide sequence of the probable causative agent of AIDS, lymphadenopathy-associated virus (LAV), has been determined. The deduced genetic structure is unique: it shows, in addition to the retroviral gag, pol, and env genes, two novel open reading frames we call Q and F. Remarkably, Q is located between pol and env and F is half-encoded by the Ur

element of the LTR. These data place LAV [HIV] apart from the previously characterised family of human T cell leukaemia/lymphoma viruses", HTLV-I and HTLV-II.²⁷

Gallo's team wrote: "The complete nucleotide sequence of two human T-cell leukaemia type III (HTLV-III) [HIV] proviral DNAs (the cDNA integrated into the cellular genome) each have four long open reading frames, the first two corresponding to the gag and pol genes. The fourth open reading frame encodes two functional polypeptides, a large precursor of the major envelope glycoprotein and a smaller protein derived from the 3'-terminus long open reading frame analogous to the long open reading frame (lor) product of HTLV-I and -II...The HTLV-III provirus is 9,749 base long".²⁸

In 1990 the HIV genome was said to consist of ten genes.²⁹ In 1996 Montagnier reported that HIV possesses eight genes³⁰ while Barre-Sinoussi reported HIV as having nine genes.³¹

However neither Montagnier's nor Gallo's team nor anybody else since has proven that the proteins which in sucrose density gradients band at the density of 1.16 g/ml and react with AIDS patients sera, (that is, the protein which they claimed were HIV proteins), are coded by these genes.

From the very beginning, sequence differences were reported between "HIV" clones. "Sequences from different clones of HTLV-III allow an analysis of the level of sequence diversity of the virus. A comparison of clones BH8 and BH5 with BH10 demonstrates a 0.9% base pair polymorphism in the coding regions of the genome and a 1.8% base pair polymorphism in the non-coding regions...The resultant differences in viral protein function remains to be determined...Diversity among different HTLV-III isolates seems to be greater than that between different HTLV-I isolates. Different isolates of HTLV-III comprise a spectrum of closely to distantly related viruses.²⁸ It soon became obvious that there is an "extraordinary scale of HIV variation".^{32,33} This fact raises several problems. How is it possible, as currently accepted:

- (a) that the proteins of all the "HIV-I" variants perform the same function? (In 1985 Gallo did not exclude the possibility that even "a 0.9% base pair polymorphism" may lead to "differences in viral protein function"?²⁸)
- (b) to prove infection with the different HIV-1 variants by serological tests using the same antigens in a universal antibody test?
- (c) to define HIV-1 infection in molecular terms? (As far back as 1989 and 1992 researchers from the Pasteur Institute concluded: "the task of defining HIV infection in molecular terms will be difficult"),^{34,35}
- (d) to produce an "HIV-1" vaccine?

THE "HIV" GENOME IN AIDS PATIENTS

If the "HIV RNA" stretch is the genome of a unique exogenous virus which infects individuals with AIDS or those at risk, then this RNA (or cDNA), the proviral DNA, should be present in fresh tissue from all these individuals and in nobody else. Furthermore, if in these individuals there is massive HIV infection, as the HIV experts claim,^{36,37} standard Southern blot hybridisation should be more than sufficient to detect it. The first such study was conducted by Gallo and his colleagues in 1984. Summarising their finding they wrote, "as shown herein, HTLV-III DNA is usually not detected by standard Southern Blotting hybridisation...when it is, the bands are often

faint...the observation that HTLV-III sequences are found rarely, if at all, in peripheral blood mononuclear cells, bone marrow, and spleen provides the first direct evidence that these tissues are not heavily or widely infected with HTLV-III in either AIDS or ARC".³⁸ These studies were confirmed by other researchers. The finding of positive hybridisation "faint", "low signal" bands was interpreted as either a very low concentration of HIV or non-specific hybridisation with other retroviruses such as HTLV-I or HTLV-II. However in 1994 Gallo admitted "We have never found HIV DNA in the tumour cells of KS [Kaposi's sarcoma]...In fact we have never found HIV DNA in T-cells".³⁹ Positive hybridisation findings, however, have been reported in non-HIV infected tissues, including the following:

- (a) although it is no longer accepted that HIV is transmitted by or is present in insects, in 1986 researchers from the Pasteur Institute found "HIV" DNA sequences in tsetse flies, black beetles and ant lions from Zaire and the Central African Republic;⁴⁰
- (b) in 1985 Weiss and his colleagues reported the isolation from two patients with common variable hypogammaglobulinaemia, a retrovirus which "was clearly related to HTLV-III/LAV". The evidence included, particles "morphologically indistinguishable from HTLV-III/LAV" in EM of cultures, positive WB with AIDS sera and hybridisation with HIV probes;⁴¹
- (c) DNA extracted from thyroid glands from patients with Grave's disease hybridises with "the entire gag p24 coding region" of HIV.⁴²

Since the late 1980s there have been many reports of the detection of the "HIV" genome using PCR. With this technique, as Kary Mullis its discoverer points out, "Beginning with a single molecule, PCR can generate 100 billion similar molecules in an afternoon".⁴³ However, "a striking feature of the results obtained" by 1990 with PCR as with the standard Southern/Northern hybridisation, was "the scarcity or apparent absence of viral DNA in a proportion of patients".⁴⁴ In the 1990s researchers from the Department of Genetics University of Edinburgh introduced a modified version of PCR, the double PCR method or nested PCR, with which they "measured provirus frequencies in infected individuals down to a level of one molecule per 10⁵ PBMCs..." (PBMC = peripheral blood mononuclear cells). They concluded "The most striking feature of the results is the extremely low level of HIV provirus present in the circulating PBMC in most cases".⁴⁴

Furthermore, HIV experts themselves present cogent reasons why the PCR cannot be used to prove HIV infection. In 1989, discussing their studies on human retroviruses, researchers from the University of New York wrote, "Irrespective of the origin of human retroviruses, their presence leads to both practical and theoretical concerns. Presently, the major practical concern is that effective use of PCR as a screening procedure for HTLV-I, HTLV-II and HIV infections must always include appropriate controls to ensure that no endogenous sequences contribute to positive signals. As previously noted, HIV unique primers corresponding to the highly conserved reverse transcriptase region...function well in the PCR amplification of HeLa DNA [a non-HIV-infected neoplastic laboratory cell line] even at annealing temperatures around 60°C".⁴⁵ In an article where he discusses the laboratory diagnosis of "HIV infection", Philip Mortimer wrote: "Other diagnostic methods, e.g. p24 antigen testing, and proviral DNA and RNA amplification exist, but these innovations in HIV diagnosis need to be matched against the anti-HIV [antibody] test and should be rejected unless they fulfil a need that antibody testing fails to meet".⁴⁶

According to another British researcher, "Those laboratories which undertake HIV screening and confirmation assays understand fully the technical problems

associated with PCR and other amplification assays and it is precisely for those reasons that PCR is NOT used as a confirmatory assay"⁴⁷ (emphasis in original). Others agree but for reasons other than mere "technical problems". As researchers from several institutions from the USA pointed out in 1996: "To evaluate the sensitivity and specificity of PCR, investigators must ascertain whether study participants are infected with HIV. Typically, a new test is compared with a superior reference (or gold standard) test...The lack of an appropriate reference test (for HIV PCR) substantially complicates evaluation". Even if a gold standard did exist, the specificity of the PCR still could not be determined for the simple reason, that this test, as the same researchers point out, is not standardised" "The criteria for determining when PCR gave positive results varied among the studies".⁴⁸ Even when the lack of standardisation is ignored and totally unsuitable gold standards are used, such as the antibody test, the specificity of PCR varies from 40 to 100% leading researchers from different institutes in the USA to conclude "Our investigation produced two main findings. First, the false-positive and false-negative rates of PCR that we determined are too high to warrant a broader role for PCR in either routine screening or in the confirmation of diagnosis of HIV infection. This conclusion is true even for the results reported from more recent, high-quality studies that used commercially available, standardised PCR assays...We did not find evidence that the performance of PCR improved over time".⁴⁸

In regard to the HIV viral load tests which are used to quantitate "HIV" in plasma, researchers from the Massachusetts School of Medicine express the problem concisely: "Plasma viral [RNA] load tests were neither developed nor evaluated for the diagnosis of HIV infection...Their performance in patients who are not infected with HIV is unknown" and their use leads to "Misdiagnosis of HIV infection". Researchers from the Service of Infectious Diseases, Institute de Salud Carlos III, are even more decisive: "since their specificity is not well known, these tests must not be used for diagnostic purposes".^{49,50} According to manufacturer Roche, "The Amplicor HIV-I [RNA] Monitor test is not intended to be used as a screening test for HIV-I or as a diagnostic test to confirm the presence of HIV-I infection" (Roche Diagnostic Systems, 06/96, 13-08088-001. Packet Insert).

In the CDC 2000 Revised AIDS Surveillance Definition, it is stated: "In adults, adolescents and children infected by other than perinatal exposure, plasma viral RNA nucleic acid tests should NOT be used in lieu of licensed HIV screening tests (e.g. repeatedly routine enzyme immunoassay) (emphasis in original).⁴⁸ The question is how can one and the same test for one and the same virus "NOT" be used to prove infection in adults, adolescents and in children infected by means other than mother-to-child transmission — but can be used to prove the latter?

CONCLUSION

Nowhere in the scientific literature is there proof of the existence of the HIV genome based upon extraction of RNA from purified retroviral particles. Instead, from the sucrose density band of 1.16 g/ml, the density at which retroviruses band, fragments of poly (A) RNA were chosen and defined as the HIV genome. However, since: (i) no proof was given that the 1.16 g/ml band contained any retrovirus particles pure or impure; (ii) poly (A) RNA is not specific to retroviruses and is found in all cells; (iii) cellular fragments containing poly (A) RNA band at the 1.16 g/ml band; (iv) culture conditions may induce the appearance of novel RNAs; then clearly there are grounds to question the proof for the existence of the HIV genome.

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[BACK](#)