Reagan Reinterprets the ABM Treaty

On more than one occasion over the past decade, according to former U.S. Ambassador Gerard Smith, the Defense Department has argued that the development and testing of exotic missile defenses are not constrained by the SALT I treaty. Until recently, Smith says, "when they heard the argumentation on the other side, they gave up."

Several months ago, however, the Pentagon did not give up. It hired a lawyer who insisted that an exotic missile defense—such as that envisioned in the "Star Wars" program—could indeed be developed and tested without constraint. Propelled through the interagency process by assistant secretary of defense Richard Perle, a committed SALT I opponent, this time the claim won official endorsement, with the consequence that dozens of doors have been effectively unlocked for "Star Wars" scientists.

The Administration's decision, which has rankled the arms control community, effectively reinterprets a key provision of SALT I, known as Article 5. It states that "each party undertakes not to develop, test, or deploy ABM [antiballistic missile] systems or components which are sea-based, air-based, space-based, or mobile land-based." Until a few weeks ago, the prevailing U.S. view—as expressed in both Pentagon and White House statements—was that this limited "Star Wars" research to lab work and tests involving ABM subcomponents. "This reading of the Treaty is plausible, but it is not the only reasonable reading," says Abraham Sofaer, the State Department's chief legal counsel.

After a fresh look at the classified negotiating record, Sofaer concluded that this provision refers only to ABM systems and components that were "current" at the time the treaty was written. New technologies, such as those presently under investigation for "Star Wars," are said to be governed by a different provision, Agreed Statement D, which clearly bans deployment but says nothing about development and testing.

This reading of the treaty is complicated by the fact that the United States clearly *tried* to obtain development and testing restrictions on exotic technologies—a point that the Administration concedes. Its new interpretation thus rests on the assertion that the United States failed to get the Soviets' agreement. The evidence is not that the Soviets actively disagreed but that they explicitly failed to signal their assent, Sofaer says. "In effect, because the Soviets succeeded in avoiding a broad binding commitment . . . we cannot properly be said to be bound by such a commitment," he argues.

Not surprisingly, this view angers Albert Carnesale, a professor of government at Harvard who served as a special adviser to the SALT I delegation. "Having been through the negotiations myself, having been on the [relevant] subgroup there, my understanding of the treaty has always been invariant: Article 5 means what it says, and prohibits development and testing regardless of the nature of the technology," he says.

Carnesale and three other former members of the U.S. delegation, Gerard Smith, Raymond Garthoff, and John Rhinelander, all remember that the Soviets initially resisted any constraints on future technologies. But they insist that the constraints were eventually accepted, even if the Soviets did not say so explicitly at the time. "It never occurred to anyone on either side to make an explicit statement," Garthoff says, because the provision's meaning appeared so obvious. In any event, the Soviets said that they accepted the constraint earlier this year.

As a result of the Administration's new interpretation, the Pentagon can legally orbit free electron lasers, kinetic kill vehicles, railguns, neutral particle beams, and other exotic technologies under the rubric of an elaborate test program. It could also "transfer" these technologies to other countries, who are not bound by a deployment ban. The only remaining constraint is that of politics, for the Reagan Administration, acting in response to protests from European allies, decided not to take advantage of the new interpretation as yet, and to keep to its original research plan. Paul Nitze, the senior U.S. arms control adviser, says that "there is no intention to deviate" from this plan, but Richard Perle says, "it remains to be seen."

—R. JEFFREY SMITH

Rumors of China-Iran Trade Clouds Nuke Pact

Legislation sponsored by Senator John Glenn (D–Ohio) to address weaknesses in the pending nuclear trade agreement with China appears to be gaining momentum in the Senate. Concern in Washington about flaws in the nuclear trade pact have been heightened in recent days by allegations that China may be doing business with Iran.

Questions about the Iranian connection, which was disclosed by Senator Alan Cranston (D–Calif.) in a statement on the Senate floor on 21 October, have not been answered fully. It has rekindled doubts about the "Agreement for Nuclear Cooperation," which President Reagan signed 24 July. Cranston's charges, which allegedly can be substantiated by intelligence reports, follow a series of House and Senate hearings that have focused on China's past nuclear trade practices and on the vagueness of proliferation safeguards assurances contained in the trade pact.

The Glenn legislation, which still must come before the Foreign Relations Committee for markup, has attracted the support of Senator Dave Durenberger (R–Minn.), chairman of the select Committee on Intelligence. The bill (S. 1754) would require that before any U.S. nuclear fuel or technology transactions proceed, China must verify that its export procedures comply with International Atomic Energy Agency rules, and recognize that the United States is not bound to okay future reprocessing requests or alterations of materials and technology. Enactment of the nuclear pact is necessary for American firms to compete against European companies to supply nuclear reactor components and engineering services to China.

Whether China has any substantive dealings with Iran is unclear. Ali Akbar Hashemi Rafsanjani, the speaker of Iran's parliament, visited China in late June. The Chinese are thought to have made a pledge then to assist Iran in the application of nuclear technology for peaceful industrial purposes. Senate sources say this information was first reported by the British Broadcasting Corporation on 4 July. A similar report subsequently appeared

Science, said they asked for three things: reissuance of the patent, with the Pasteur Institute as a coholder; recognition of the fact that the Pasteur group was the first to discover the virus and apply for a patent; and an agreement that the Genetic Systems test kit could be marketed without hindrance.

The HHS officials asked Dedonder to supply details of the Pasteur Institute's version of the events. This was done in a memorandum dated 16 August, which laid out the institute's case for declaring the U.S. patent invalid. According to Dedonder, "HHS said that the documents are not sufficient to change their position."

The Pasteur Institute has therefore been pursuing its case directly with the Patent Office. According to Dedonder, the institute is seeking what is called an "interference," which, if granted, would essentially mean that the Patent Office would reopen consideration of the U.S. patent and the Pasteur Institute would be given a year to prove its case. During the interference period, the patent would not be enforceable and thus Genetic Systems would be able to market its test kit. Failing that, "If there is no solution, then we will have to go to court," says Dedonder.

The Pasteur Institute's memorandum, a copy of which has been obtained by *Science*, sets out two chief grounds on which the institute is likely to challenge the U.S. patent. First, "the Institut Pasteur can establish that its team had all the essential elements of the subject matter of the Gallo *et al.* patent, prior to any patent filing of Gallo." Second, "the subject matter of the Gallo *et al.* patent was obtained or derived from the Montagnier team."

The first argument rests on the fact that the Pasteur group had isolated a retrovirus early in 1983 and determined that it was different from HTLV-I and HTLV-II, the only other known human retroviruses. A paper describing this work was published in the 20 May 1983 issue of *Science*. The Pasteur group had also constructed an ELISA test using proteins from this viral isolate as antigens, and employed the test in serological studies establishing a link between the virus and AIDS. "There is a prima facie case that the Montagnier team was 'first,' " the Pasteur memo states.

The basis for the second argument is a series of exchanges between the Montagnier and Gallo groups, through which, the Pasteur memo contends, Gallo gained an advantage. These include the fact that Montagnier sent Gallo a prepublication copy of a his *Science* paper, a presentation Montagnier made at a meeting at NCI in July 1983, and a paper Montagnier delivered at a meeting at Cold Spring Harbor in September, which included preliminary serological findings from use of the ELISA test.

The messiest part of this second argument concerns a sample of supernatant containing a small amount of LAV that Montagnier sent to Gallo on 23 September 1983. The Pasteur memo notes that when the precise genetic sequences of HTLV-III and LAV were determined early this year, the two were remarkably similar, while the sequences of other isolates have turned out to be quite different.



The French virus

Reagents supplied by Gallo helped determine that it was not HTLV-I or HTLV-II.

By implication, the memorandum suggests that Gallo's group somehow grew the French isolate. "The Institut Pasteur can establish a prima facie case of breach of contract in that the retrovirus given to [Gallo's group] or one derived therefrom to the best of Institut Pasteur's knowledge, was used in contravention of the terms of the letter agreement," which restricted use of the isolate to research purposes, the memo states.

Gallo indignantly disputes this allegation on several counts, including the fact that the viruses are not identical and that the amount of virus Montagnier sent would not have been sufficient to infect a cell line (see box on page 643).

Although federal officials are reluctant to discuss in detail the legal aspects of the dispute with the Pasteur Institute, they challenge some of the scientific claims. For example, they point out that although it is certainly true that the Pasteur group was the first to identify the correct virus in the literature, Gallo's group was also getting glimpses of a new retrovirus as early as December 1982 but could not grow it or properly characterize it.

Many scientists also found the Pasteur group's serological studies less than convincing in demonstrating that LAV is the cause of AIDS. In their patent application, for example, the Pasteur researchers note that they detected LAV antibodies in only 20 percent of serum samples of AIDS patients, and early in 1984, they reported that this figure had risen to only 37.5 percent. Moreover, federal officials point out that the Pasteur Institute's patent application specifically states that LAV's "envelope proteins are not detected immunologically by the sera of LAS [lymphadenopathy syndrome] and AIDS afflicted patients." The envelope protein is, in fact, the most immunogenic viral antigen.

Federal officials also point out that the flow of information and materials was not all one way. For example, the Pasteur group would not have been able to determine that their first isolate was different from HTLV-I without reagents from Gallo. Finally, they argue that it was Gallo's breakthrough in mass-producing the virus that enabled ELISA tests to be produced on a scale large enough to be useful for monitoring blood donations.

The unfortunate aspect of this patent dispute is that it is being cast as a winner-take-all contest. In fact, both groups, although moving along somewhat separate tracks, made important contributions.

For example, it was largely the prior work of Gallo and his many collaborators that laid the groundwork for searching for a retrovirus and, when it was isolated, determining that it was different from previous human retroviruses. As one of Gallo's collaborators puts it, "If this disease had appeared 10 years ago, we would be completely lost." Gallo's group also achieved the key breakthrough of finding a cell line that would produce the virus without dying off, which in turn led to the rapid development of a sensitive ELISA test and convincing serological evidence that the virus is the cause of AIDS.

The Pasteur group was the first to identify the correct virus in the literature and to recognize the virus's propensity for killing the cells it infected. By careful work with small quantities of the virus, they were able over the following year to link their virus more firmly to the disease. And they also have the advantage of being the first to file for a patent on the ELISA test.

As Nowinski of Genetic Systems puts it, "It is pretty remarkable what both Montagnier and Gallo have done."

—COLIN NORMAN

Patent Dispute Divides AIDS Researchers

A tussle between the Pasteur Institute and the U.S. government over rights to blood test kits rests on issues of scientific priority

In April 1984, rumors began circulating around the scientific community that Robert C. Gallo of the National Cancer Institute (NCI) had made a major breakthrough in the search for the cause of acquired immune deficiency syndrome (AIDS). Gallo had in fact been playing his cards close to his chest since early November, as he and his many co-workers accumulated a mass of data firmly implicating a newly discovered retrovirus as the cause of the disease.

Gallo was about to report, in four papers in *Science**, that his group had isolated a virus, which he called HTLV-III (for human T-lymphotropic virus type III), from 48 patients with AIDS and AIDS-related symptoms. The papers also described a system for mass-producing the virus—something that had eluded researchers for more than a year—and reported the detection of antibodies to HTLV-III in the blood of the vast majority of AIDS patients he tested, but in only one of 186 healthy controls.

To most people, the evidence was convincing, and Secretary of Health and Human Services Margaret Heckler scheduled a press conference on 23 April to tell the world.

The day before Heckler's big announcement, however, an article appeared on the front page of the *New York Times* stating that a team of researchers at the Pasteur Institute in Paris had discovered the cause of AIDS. The article was based largely on an interview with James O. Mason, the head of the Centers for Disease Control (CDC) in Atlanta, which had been cooperating closely with the French group. Mason said that data gathered over the previous few weeks had provided strong evidence that a virus first isolated by the Pasteur group early in 1983 was the AIDS agent. The French called their virus lymphadenopathy-associated virus, or LAV.

To Gallo and his colleagues, Mason's announcement looked like a deliberate attempt by CDC and the Pasteur group to steal his thunder. Relations between Gallo and CDC were already strained, and Gallo was competing with the French researchers to nail down the cause of AIDS. Mason says, however, that he had not seen Gallo's papers when he spoke with the *New York Times*, and Pasteur officials deny any part in Mason's announcement.

The Pasteur group, which was headed by Luc Montagnier, Jean Claude Chermann, and Françoise Barré-Sinoussi, were annoyed when they subsequently read Gallo's papers, however, because they felt he had slighted their contributions. Gallo noted that the French team had reported isolating LAV, but said that the virus had not been sufficiently characterized to know whether it was the same as HTLV-III. "I was shocked by the way he presented our data," Montagnier says.

The Pasteur group, working with limited quantities of LAV, had done considerable characterization of the virus, found that it selectively infects a class of lymphocytes known as T4 cells—key components of the immune system that are missing or severely depleted in AIDS patients—and developed a test to detect LAV antibodies in blood. Their research had, however, been hampered by the fact that they could not grow the virus in quantity because it killed the cells it infected.

Gallo had solved this problem by infecting a line of T cells established from a leukemia patient with virus he had isolated from AIDS patients. The cells produced large quantities of virus but did not die off. This break-through enabled Gallo's group to mass produce the virus, characterize it in detail, and develop a sensitive assay to detect HTLV-III antibodies. It also led directly to the commercial development of a test for mass screening of blood donations. (For a detailed description of the work of both groups, see part 1 of this article in last week's issue.)

The ill feeling surrounding the publication of Gallo's papers exacerbated a scientific dispute that was already dividing the two groups over the name and classification of the AIDS virus. The dispute gathered momentum early this year when the genetic sequences of HTLV-III, LAV, and a third virus isolated by Jay Levy of the University of California at San Francisco, were published. All three isolates are clearly variants of the same virus, but they differ substantially from other members of the HTLV family (see box on p. 641).

In addition to the contests over priority and nomenclature, another issue has come to dominate relations between the two groups. It involves patent rights on kits that are being used commercially to test blood samples for antibodies to the AIDS virus. Although the dispute is formally between the Pasteur Institute and the U.S. government, it is intricately linked with the question of priorities.

At issue is a patent, applied for by the U.S. government on 23 April 1984 and awarded on 28 May 1985, covering a method of detecting HTLV-III antibodies by exposing serum samples to proteins from the virus. If antibodies are present in the serum, they will bind to the viral antigens to form complexes that can be detected by various techniques,

The War on AIDS

This is the second part of a two-part article on the discovery and identification of the AIDS virus, and the third piece in a series on AIDS research.† Future articles will examine the epidemiology of the disease and research on vaccines and therapy.



Raymond Dedonder

"If there is no solution, then we will have to go to court."

**Science*, 4 May 1984, pp. 497–508.

†Previous articles in the series were published in *Science*, 25 October, p. 418, and 1 November, p. 518.

THE PATENT OFFICE,
25 SOUTHAMPTON BUILDINGS,
LONDON.

TOP SECRET

OFFICIAL USE ONLY

1. Dates?
2. Report of visit?

I, the undersigned, being an officer duly authorised in accordance with Section 62(3) of the Patents and Designs Act 1907, to sign and issue certificates on behalf of the Comptroller-General, hereby certify that annexed hereto is a true copy of the documents as originally filed in connection with the patent application identified therein.

In accordance with the Patents (Companies Re-registration) Rules 1982, if a company named in this certificate and any accompanying documents, has re-registered under the Companies Act 1980 with the same name as that with which it was registered immediately before re-registration save for the substitution as, or the inclusion as, the last part of the name of the words "public limited company" or their equivalents in Welsh, references to the name of the company in this certificate and any accompanying documents shall be treated as references to the name with which it is so re-registered.

In accordance with the rules, the words "public limited company" may be replaced by p.l.c., plc, P.L.C. or PLC.

Please turn over

Re-registration under the Companies Act does not constitute a new legal entity but merely subjects the company to certain additional company law rules.

Witness my hand this
day of SEPTEMBER 198

25 [illegible]
London, WC2A 1AY

Fee: £10.00 1983 24800

REQUEST FOR GRANT OF A PATENT

2324800

THE GRANT OF A PATENT IS REQUESTED BY THE UNDERSIGNED ON THE BASIS OF THE PRESENT APPLICATION

I Agent's Reference KEG/25921

II Title of Invention Antigens,means and method for the diagnosis of lymphadenopathy and acquired immune deficiency syndrome

III Applicant or Applicants *(See note 2)*

Name (First or only applicant) INSTITUT PASTEUR

Country FRANCE State ADP Code No.

Address 25-28 rue du Dr.Roux, 75015 Paris, France

Name (of second applicant, if more than one)

Country State

Address

IV Inventor (see note 3)

(a) ~~The applicant(s) is/are the sole/joint inventor(s)~~

or

(b) A statement on Patents Form No. 7/77 4/will be furnished

V Name of Agent (if any) *(See note 4)* Reddie & Grose | ADP CODE NO

VI Address for Service (See note 5) 16 Theobalds Road
London WC1X 8PL

VII Declaration of Priority (See note 6)

Country	Filing date	File number

VIII The Application claims an earlier date under Section 8(3), 12(6), 15(4), [illegible](4) (See note 7)

Section No.

Earlier application or patent number and filing

2	Description	22 Sheet(s)	2	Translation of priority document	NO
3	Claim(s)	2 Sheet(s)	3	Request for Search	NO
4	Drawing(s)	1 Sheet(s)	4	Statement of Inventorship and Right to Apply	NO
5	Abstract	1 Sheet(s)	5		

X It is suggested that Figure No 1 of the drawings (if any) should accompany the abstract when published.

XI Signature *(See note 8)*

Reddie & Grose, Agents for the Applicant(s)

NOTES:

1. This form, when completed, should be brought or sent to the Patent Office together with the prescribed fee and two copies of the description of the invention, and of any drawings.

2. Enter the name and address of each applicant. Names of individuals should be indicated and the surname or family name should be underlined. The names of all partners in a must be given in full. Bodies corporate should be designated by their corporate name and the count , of incorporation and, where appropriate, the state of incorporation within that country should be entered where provided. Full corporate details, eg "a corporation organised and existing under the laws of the State of Delaware, United States of America," trading styles, eg "trading as xyz company", nationality, and former names, eg "formerly [known as] ABC Ltd." are not required and should not be given. Also enter applicant(s) ADP Code No. (if known).

3. Where the applicant or applicants is/are the sole inventor or the joint inventors, the declaration (a) to that effect at IV should be completed, and the alternative statement (b) deleted. If, however, this is not the case the declaration (a) should be struck out and a statement will then be required to be filed upon Patent Form No 7/77.

4. If th licant has appointed an agent to act on his behalf, the agent's name and the address of his place of busine hould be indicated in the spaces available at V and VI. Also insert agent's ADP Code No. (if known) in the box provided.

5. An address for service in the United Kingdom to which all documents may be sent must be stated at VI. It is recommended that a telephone number be provided if an agent is not appointed.

6. declaration of priority at VII should state the date of the previous filing and the country in which it was made and indicate the file number, if available.

7. When an application is made by virtue of section 8(3), 12(6), 15(4), or 37(4) the appropriate section should be identified at VIII and the number of the earlier application or any patent granted thereon identified.

8. Attention is directed to rules 90 and 105 of the Patent Rules 1982.

9. Attention of applicants is drawn to the desirability of avoiding publication of inventions relating to any article, material or device intended or adapted for use in war (Official Secrets Acts, 1911 and 1920). In particular, where an application for a patent has been filed at the Patent Office the comptroller will consider whether publication or communication of the invention should be prohibited or restricted under section 22 of the Act and will inform the applicant if such prohibition is necessary.

10. Applicants resident in the United Kingdom are also reminded that, under the provisions of section 23 applications may not be filed abroad without written permission or unless an application has been filed not less than six weeks previously in the United Kingdom for a patent for the same invention and no direction prohibiting publication or communication has been given or any such direction has been revoked.

The invention relates to antigens, means and methods for the diagnosis of lymphadenopathy and acquired immune deficiency syndrome.

The acquired immune deficiency syndrome (AIDS) has recently been recognized in several countries. The disease has been reported mainly in homosexual males with multiple partners, and epidemiological studies suggest horizontal transmission by sexual routes as well as by intravenous drug administration, and blood transfusion. The pronounced depression of cellular immunity that occurs in patients with AIDS and the quantitative modifications of subpopulations of their T lymphocytes suggest that T cells or a subset of T cells might be a preferential target for the putative infectious agent. Alternatively, these modifications may result from subsequent infections. The depressed cellular immunity may result in serious opportunistic infections in AIDS patients, many of whom develop Kaposi's sarcoma. However, a picture of persistent multiple lymphadenopathies has also been described in homosexual males and infants who may or may not develop AIDS. The histological aspect of such lymph nodes is that of reactive hyperplasia. Such cases may correspond to an early or a milder form of the disease.

It has been found that one of the major etiological agents of AIDS and of lymphadenopathy syndrom (LAS), which is often considered as a prodromic sign of AIDS, should consist of a T-lymphotropic retrovirus which has been isolated from a human tumor or a homosexual patient with multiple lympha-denopathies. The virus appears to be distinc om the human

[illegible] 69 (No. 6), 1209 (1982)].

[illegible] has been known as belonging to the so-called HTLV-1 subgroup.

The patient was a 33-year-old homosexual male who sought medical consultation in December 1982 for cervical lymphadenopathy and asthenia (patient 1). Examination showed axillary and inguinal lymphadenopathies. Neither fever nor recent loss of weight were noted. The patient had a history of several episodes of gonorrhea and had been treated for syphilis in September 1982. During interviews he indicated that he had had more than 50 sexual partners per year and had travelled to many countries, including North Africa, Greece, and India. His last trip to New York was in 1979.

Laboratory tests indicated positive serology (immunoglobulin G) for cytomegalovirus (CMV) and Epstein-Barr virus. Herpes simplex virus was detected in cells from his throat that were cultured on human and monkey cells. A biopsy of a cervical lymph node was performed. One sample served for histological examination, which revealed follicular hyperplasia without change of the general structure of the lymph node. Immunohistological studies revealed, in paracortical areas, numerous T lymphocytes ($OKT3^+$). Typing of the whole cellular suspension indicated that 62 percent of the cells were T lymphocytes ($OKT3^+$), 44 percent were T-helper cells ($OKT4^+$), and 16 percent were suppressor cells ($OKT8^+$).

Cells of the same biopsed lymph node were put in culture medium with phytohemagglutinin (PHA), T-cell growth factor (TCGF), and antiserum to human α interferon. The cells were grown in RPMI-1640 medium supplemented with antibiotics; 10^{-5} M β-mercaptoethanol, 10 percent fetal calf serum, 0.1 percent sheep antibody to human α interferon (neutralizing titer, 7 IU at 10^{-5} dilution and 10 percent TCGF, free of PHA. The reason for using the antiserum

[redacted] chronically infected by [redacted] retroviruses. In the mouse system, it had previously been shown that anti-serum to interferon could increase retrovirus production by a factor of 10 to 50 - F. Barré-Sinoussi et al., "Ann. Microbiol. (Institut Pasteur)" 130B, 349 (1979). After 3 days, the culture was continued in the same medium without PHA. Samples were regularly taken for reverse transcriptase assay and for examination in the electron microscope.

After 15 days of culture, a reverse transcriptase activity was detected in the culture supernatant by using the ionic conditions described for HTLV-I (B.J. Poiesz et al. "Proc. Natl. Acad. Sci. U.S.A." 77, 7415 (1980)). Virus production continued for 15 days and decreased thereafter, in parallel with the decline of lymphocyte proliferation. Peripheral blood lymphocytes cultured in the same way were consistently negative for reverse transcriptase activity, even after 6 weeks. Cytomegalovirus could be detected, upon prolonged cocultivation with MRC5 cells, in the original biopsy tissue, but not in the cultured T lymphocytes at any time during culture.

The invention relates to the newly isolated virus as a source of the above said antigen which will be defined later.

The newly isolated virus, which will hereafter be termed as LAV$_1$, will however be described first.

The virus is transmissible to cultures of T lymphocytes obtained from healthy donors. Particularly virus t[redacted]ission was attempted with the use of a culture of [redacted] culture of T lymphocytes established from an adult healthy [redacted] healthy donor of the Blood Transfusion Center at the Pasteur Institute. On day 3, half of the culture was co-

[illegible] could be detected in the supernatant on day 13 of the coculture but was not detectable on days 5 and 10. The reverse transcriptase had the same characteristics as that released by the patient's cells and the amount released remained stable for 15 to 20 days. Cells of the uninfected culture of the donor lymphocytes did not exhibit reverse transcriptase activity during this period or up to 6 weeks when the culture was discontinued.

The cell-free supernatant of the infected coculture was used to infect 3-day-old cultures of T lymphocytes from two umbilical cords, LC1 and LC5, in the presence of Polybrene (2 µg/ml). After a lag period of 7 days, a relatively high titer of reverse transcriptase activity was detected in the supernatant of both cord lymphocyte cultures. Identical cultures, which had not been infected, remained negative. These two successive infections clearly show that the virus could be propagated on normal lymphocytes from either new-borns or adults.

In the above co-cultures one used either the cells of patient 1 as such (they declined and no longer grew) or cells which had been pre-X-rayed or mitomycin C-treated.

The LAV_1 virus, or LAV_1 virus suspensions, which can be obtained from infected cultures of lymphocytes have characteristics which distinguish them completly from other HTLV. These characteristics will be referred to hereafter and, when appropriate, in relation to the

[illegible] shows curves representative of variation of reverse transcriptase activity and $[^3H]$uridine activity respectively versus successive fractions of the LAV virus in the [illegible] sucrose gradient, after ultracentrifugation [illegible] of the virus contents of a cell-free supernatant obtained from a culture of infected lymphocytes.

[illegible] according to the procedure which was used in relation to virus from patient 1. The results of the analysis are illustrated in Fig. 1. Cord blood T lymphocytes infected with virus from patient 1 were labelled for 18 hours with [^{3}H]uridine (28 Ci/mmole, Amersham ; 20 µCi/ml). Cell-free supernatant was ultracentrifuged for 1 hour at 50,000 rev/min. The pellet was resuspended in 200 µl of NTE buffer (10 mM tris, pH 7.4, 100 mM NaCl, and 1 mM EDTA) and was centrifuged over a 3-ml linear sucrose gradient (10 to 60 percent) at 55,000 rev/min for 90 minutes in an IEC type SB 498 rotor . Fractions (200 µl) were collected, and 30 µl samples of each fraction were assayed for DNA RNA dependant polymerase activity with 5 mM Mg^{2+} and poly(A)-oligo-$(dT)_{12-18}$ as template primer ; a 20-µl portion of each fraction was precipitated with 10 percent trichloroacetic acid and then filtered on a 0.45-µm Millipore filter. The ^{3}H-labelled acid precipitable material was measured in a Packard β counter.

That the new virus isolate was a retrovirus was further indicated by its density in the above sucrose gradient, which was 1.16, and by its labelling with [^{3}H]uridine (fig. 1). A fast sedimenting RNA appears to be associated with the LAV_1 virus.

Virus-infected cells from the original biopsy as well as infected lymphocytes from the first and second viral passages were used to determine the optimal requirements for reverse transcriptase activity and the template specificity of the enzyme. The results were the same in all instances. The reverse transcriptase activity displayed a strong affinity for poly(adenylate-oligodeoxythymidylate)[poly(A)-oligo$(dT)_{12-18}$], and required Mg^{2+} with

[redacted] by [redacted]n D. This [redacted], as well as the preferential specificity for riboseadenylate-deoxythymidylate over deoxyadenylate-deoxythymodylate, distinguish the viral enzyme from DNA-dependent polymerases.

Electron microscopy of ultrathin sections of virus-producing cells shows two types of particles, presumably corresponding to the immature and mature forms of the virus : immature particles are budding at the cell surface, with a dense crescent in close contact with the plasma membrane. Occasionally, some particles remain in this state, while being freed from the cell surface.

Mature particles have a quite different morphology with a small, dense, eccentric core (mean diameter: 41 nm). Most virions are round (mean diameter : 139 nm) or ovoid, but in some pictures, especially in the particles seen in the original culture from which the virus was isolated, a tailed morphology can also be observed. The latter form can also be observed in cytoplasmic vesicles which were released in the medium. Such particles are also formed by budding from vesicle membranes.

Morphology of mature particles is clearly distinct from HTLV, whose large core has a mean diameter of 92 nm.

Helper T-lymphocytes (Leu 3 cells) form the main target of the virus. In other words the LAV_1 virus has particular tropism for these cells. Leu 3 cells are recognizable by the monoclonal antibodies commercialized by ORTHO under the trademark OKT4. In contrast enriched cultures of Leu 2 cells, which are mainly suppressor or cytotoxic cells and which are recognized by the monoclonal antibodies commercialized by ORTHO under the

[redacted] any detectable RT activity even [redacted] weeks after virus infection.

In most cases of AIDS, the ratio of $OKT4^+$ over $OKT8^+$ cells which is normally over 1, is depressed to values as low as 0.1 or less.

The LAV_1 virus is also immunologically distinct from previously known HTLV-1 isolates from cultured T lymphocytes of patients with T lymphomas and T leukemias. The antibodies used were specific for the p19 and p24 core proteins of HTLV-1. A monoclonal antibody to p19 (M. Robert-Guroff et al. "J. Exp. Med." 154, 1957 (1981)) and a polyclonal goat antibody to p24 (V.S. Kalyanaraman et al. "J. Virol.", 38, 906 (1981)) were used in an indirect fluorescence assay against infected cells from the biopsy of patient 1 and lymphocytes obtained from a healthy donor and infected with the same virus. The LAV_1 virus-producing cells did not react with either type of antibody, whereas two lines of cord lymphocytes chronically infected with HTVL 1 (M. Popovic, P.S. Sarin, M. Robert-Guroff, V. S. Kalyanaraman, D. Mann, J. Minowada, R.C. Gallo, "Science" 219, 856 (1983)) and used as controls showed strong surface fluorescence.

In order to determine which viral antigen was recognized by antibodies present in the patient's sera, several immunoprecipitation experiments were carried out. Cord lymphocytes infected with virus from patient 1 and uninfected controls were labelled with [^{35}S]methionine for 20 hours. Cells were lysed with detergents, and a cytoplasmic S10 extract was made. Labelled virus released in the supernatant was banded in a sucrose gradient. Both materials were immunoprecipitated by antiserum to HTVL-1 p24, by serum from patient 1, and by serum samples from healthy donors. Immunocomplexes were

[redacted] protein present in the virus-infected cells from patient 1 and in LC1 cells infected with this virus, was specifically recognized by serum from patient 1 but not by antiserum to HTLV-1 p24 obtained under similar conditions or serum of normal donors. Conversely the p24 present in control HTLV-infected cell extracts was recognized by antibodies to HTLV but not by serum from patient 1.

The main protein (p25) detected after purification of ^{35}S-methionine-labelled virus has a molecular weight of about 25,000 (or 25K). This is the only protein recognized by the serum of patient 1. By analogy with other retroviruses, this major protein was considered to be located in the viral core.

This can be confirmed in immuno-electron microscopy experiments, which show that the patient's serum can agglutinate the viral cores. Conversely, an antiserum raised in rabbit against an ether treated virus did not precipitate the p25 protein.

The viral origin of other proteins seen in polyacrylamide gel electrophoresis of purified virus is more difficult to assess. A p15 protein could be seen after silver staining, but was much weaker after ^{35}S-methionine perhaps due to the paucity of this amino-acid in the protein. In the higher MW range, a contamination of the virus by cellular proteins, either inside or outside the viral envelope, is likely. A 36K and a 42K protein and a 80K protein were constantly formed to be associated with the purified virus and may represent the major envelope proteins.

No p 19 (having a molecular weight of about 19 mM) was isolated from LAV, extracts.

[redacted]

[redacted] of patients afflicted with LAS or AIDS. Needless say any type of immunological assay may be brought into play. By way of example immunofluorescence or immunoenzymatic assays or radio-immunoprecipitation tests are particularly suitable.

As a matter of fact and except under exceptional circumstances, sera of diseased patients do not recognize the intact LAV_1 virus, or viruses having similar phenotypical or immunological properties. The envelope proteins of the virus appeared as not detectable immunologically by the patients' sera. However as soon as the core proteins become exposed to said sera, the immunological detection becomes possible. Therefore the invention concerns all extracts of the virus, whether it be the crudest ones - particularly mere virus lyzates - or the more purified ones, particularly extracts enriched in the p25 protein or even the purified p25 protein or in protein immunologically related therewith. Any purification procedure may be resorted to. By way of example only, one may use purification procedures such as disclosed by R.C. Montelaro et al, J. of Virology, June 1982, pp. 1029-1038.

The invention concerns more generally extracts of any virus having similar phenotype and immunologically related to that obtained from LAV_1. Sources of viruses of the LAV type consist of T-lymphocyte cultures isolatable from LAS- and AIDS - patients or from haemophiliacs.

[redacted] obtained by
[redacted] lymphocytes of the retroviruses
isolated from :

1) lymph node lymphocytes of a caucasian homosexual with multiple partners, having extensive Kaposi sarcoma lesions and severe lymphopenia with practically no $OKT4^+$ lymphocytes in his blood ;

2) blood lymphocytes of a young B haemophiliac presenting neurotoxoplasmosis and $OKT4^+/OKT8^+$ ratio of 0.1.

These two retroviruses have been named IDAV1 and IDAV2 respectively (for Immune Deficiency Associated Virus). Results of partial characterization obtained so far indicate similarity - if not identity - of IDAV1 and IDAV2 to LAV1 :

- same ionic requirements and template specificities of reverse transcriptase,
- same morphology in ultrathin sections,
- antigenically related p25 proteins : serum of LAV1 patient immunoprecipitates p25 from IDAV1 and IDAV2 ; conversely, serum from IDAV2 patient immunoprecipitates LAV1 p25.

IDAV1 patient serum seemed to have a lower antibodies titer and gave a weak precipitation band for LAV1 and IDAV1 p25 proteins. The p25 protein of IDAV1 and IDAV2 was not recognized by HTLV p24 antiserum.

These similarities suggest that all these three isolates belong to the same group of viruses.

The invention further relates to a method of <u>in vitro</u> diagnosis of LAS or AIDS, which comprises contacting a serum or other biological medium from a patient to be diagnosed ----------

[illegible] as above defined and detecting the immunological reaction.

Preferred methods bring into play immunoenzymatic or immunofluorescent assays, particularly according to the ELISA technique. Assays may be either direct or indirect immunoenzymatic or immunofluorescent assays.

Thus the invention also relates to labelled virus extracts whatever the type of labelling : enzymatic, fluorescent, radioactive, etc..

Such assays include for instance :

- depositing determined amounts of the extract according to the invention in the wells of titration microplate;
- introducing in said wells increasing dilutions of the serum to be diagnosed;
- incubating the microplate;
- washing the microplate extensively;
- introducing in the wells of the microplate labelled antibodies directed against blood immunoglobulins, the labelling being by an enzyme selected from those which are capable of hydrolysing a substrate, whereby the latter then undergoes a modification of its absorption of radiations, at least in a determined wavelength band and
- detecting, preferably in a comparative manner with respect to a control, the amount of substrate hydrolysis as a measure of the potential risk or effective presence of the disease.

The invention also relates to kits for the above-said diagnosis which comprise :

- an extract or more purified fraction of the abovesaid types of viruses, said extract or fraction being labelled such as by a radioactive, enzymatic or immunofluorescent label ;

[illegible] antibodies directed against blood immunoglobulins

[redacted] protein A advantageously fixed on a water-insoluble support such as agar beads) ;

- a lymphocyte extract obtained from a healthy person ;
- buffers and, if appropriate, substrates for the vizualization of the label.

Other features of the invention will further appear as the description proceeds of preferred isolation and culturing procedures of the relevant virus, of preferred extraction methods of an extract suitable as diagnostic means, of a preferred diagnosis technique and of the results that can be achieved.

1. VIRUS PROPAGATION :

Cultured T-lymphocytes from either umbilical cord or blood or bone marrow cells from healthy, virus negative, adult donors are suitable for virus propagation.

There is however some variation from individual to individual in the capacity of lymphocytes to grow the virus. Therefore, it is preferable to select an adult healthy donor, having no antibodies against the virus and whose lymphocytes repeatly did not release spontaneously virus, as detected by reverse transcriptase activity (RT) nor expressed viral proteins.

Lymphocytes of the donor were obtained and separated by cytophoresis and stored frozen at -180°C in liquid nitrogen, in RPMI 1640 medium, supplemented with 50 % decomplemented human serum and 10 % DMSO.

For viral infection, lymphocytes were put in culture (RPMI 1640) medium with phytohaemagglutinin (PHA) at the concentration of 5.10^6 cells/ml for 3 days.

Then, the medium was removed and cells resuspended in viral suspension (crude supernatant of virus-

[redacted] Optimal conditions [redacted] were 2.10^6 cells for 5 to [redacted] cpm of RT activity, the latter determined as previously described. After 24 hours, cells were centrifuged to remove the unadsorbed virus and resuspended in culture PHA-free medium and supplemented with PHA-free TCGF (Interleukin 2) : (0.5 - 1 U/ml, final concentration), POLYBREN (Sigma) 2 µg/ml and anti-interferon α sheep serum, inactivated at 56°C for 30 minutes (0.1 % of a serum which is able to neutralize 7 U of α.leucocyte interferon at a 1/100,000 dilution).

Virus production was tested every 3 days by RT activity determination on 1 ml samples.

The presence of anti-interferon serum is important in virus production : when lymphocytes were infected in the absence of anti-human-α-interferon serum, virus production, as assayed by RT activity, was very low or delayed. Since the sheep antiserum used was raised against partly purified α leucocyte interferon, made according to the Cantell technique, the role of components other than interferon cannot be excluded.

Virus production starts usually from day 9 to 15 after infection, and lasts for 10-15 days. In no cases was the emergence of a continuous permanent line observed.

2. VIRUS PURIFICATION :

For its use in ELISA, the virus was concentrated by 10 % Polyethylenglycol (PEG 6000) precipitation and banded twice to equilibrium in a 20-60 % sucrose gradient. The viral band at density 1.16 is then recovered and usable as such for ELISA assays.

For use in RIPA (radio-immune precipitation assay), purification in isotonic gradients of Metrizamide (sold under the trademark NYCODENZ by Nyegaard, Oslo) was found to be preferable. Viral density in such gradients was very low (1.10-1.11).

Metabolic labelling with ^{35}S-methionine of cells and virus (RIPA) followed by polyacrylamide gel electrophoresis were performed as above described, except for the following modifications for RIPA : virus purified in NYCODENZ was lysed in 4 volumes of RIPA containing 500 U/ml of aprotinin. Incubation with 5 µl of serum to be tested was made for 1 hour at 37°C and then 18 hours at +4°C. Further incubation of the immunocomplexes with protein A SEPHAROSE beads was for 3 hours at +4°C.

3. PREPARATION OF THE VIRUS EXTRACT FOR ELISA ASSAYS :

Virus purified in sucrose gradient as above described, is lysed in RIPA buffer (0.5 % SDS) and coated on wells of microtest plates (Nunc).

Preferred conditions for the ELISA assay are :

After addition to duplicate wells of serial dilutions of each serum to be tested, the specifically fixed IgGs are revealed by goat anti-human IgG coupled with peroxydase. The enzymatic reaction is carried out on ortho-phenylene-diamine as substrate and read with an automatic spectrophotometer at 492 nm.

On the same plate each serum is tested on a control antigen; a crude cytoplasmic lysate of uninfected T-lymphocytes from the same donor is used

[redacted] considered as positive (antibodies against the virus) when the difference between O.D. against the viral antigen and O.D. against control cellular antigen is at least 0.30.

Hereafter there is disclosed a specific test for assaying the above mentionned disease or exposure to disease risks.

Method.

This ELISA test is for detecting and titration of seric anti-retrovirus type LAV antibodies.

It comprises carrying out a competition test between a viral antigen (cultivated on T lymphocytes) and a control antigen constituted by a lysate of the same though non-infected lymphocytes.

The binding of the antibodies on the two antigens is revealed by the use of a human antiglobulin labelled with an enzyme which itself is revealed by the addition of a corresponding substrate.

Preparation of the viral antigen.

The cellular cultures which are used are T lymphocytes of human origin which come from :

. umbilical cord blood,

. bone marrow,

. blood of a healthy donnor.

After infection of the cells by the virus, the supernatant of the infected cell culture is used. It is concentrated by precipitating with 10 % PEG, then purified (two or three times) on a (20-60 %) sucrose gradient by ultracentrifugation to equilibrium.

The viral fractions are gathered and concentrated by centrifugation at 50 000 rotations per minute for 60 minutes.

[redacted] in a minimum volume of buffer PBS at pH 7.4 (Tris 0.01 M, NaCl 0.1 M, EDTA 0.001 M).

The proteic concentration is determined by the Lowry method.

The virus is then lysed by a (RIPA + SDS) buffer (0.5 % final) for 15 minutes at 37°C.

Preparation of the control antigen.

The non-infected lymphocytes are cultured according to the preceding conditions for from 5 to 10 days. They are centrifuged at low speed and lysed in the RIPA buffer in the presence of 5 % of the product commercialized under the name of ZYMOFREN (Spécia) (500 u/ml). After 15 minutes at 4°C with frequent stirrings with vortex, the lysate is centrifuged at 10 000 rotations per minute. The supernatant constitutes the control antigen. Its concentration in protein is measured by the Lowry method.

Reagents.

1 - Plates = NUNC - special controlled ELISA

2 - Buffer PBS : pH 7.5

3 - TWEEN 20

4 - Carbonate buffer : pH = 9.6 (CO_3Na_2 = 0.2 M

(CO_3HN_a = 0.2 M

5 - Non foetal calf serum : which is stored in frozen state (BIOPRO),

6 - Bovine serum albumin (BSA) SIGMA (fraction V)

7 - Human anti IgG (H+L) labelled with peroxydase PASTEUR, in tubes of 1 ml preserved at 4°C

8 - Washing buffer = PBS buffer, pH 7.5 + 0.05 % TWEEN 20

Dilution of the conjuguate is carried out at the dilution indicated in PBS buffer + TWEEN 20 (0.05%)+ bovine albumin 0.5 g per 100 ml

PROTEIN IS MEASURED BY THE LOWRY METHOD

bovine serum albumin per 100 ml

10 - Substrate : OPD

. Citrate buffer pH = 5.6 trisodic citrate ($C_6H_5Na_3O_7$, $2H_2O$), 0.05 M ; citric acid ($C_6H_8O_7$, $1H_2O$), 0.05 M.

. Hydrogen peroxide = at 30 % (110 volumes) - used at 0.03 % when using citrate buffer.

. Orthophenylene diamine = SIGMA 75 mg per 25 ml of buffer - which is diluted in buffer extemporaneously.

Preparation of the plates.

The plates which are used have 96 U-shaped wells (NUNC= ELISA). They include 12 rows of 8 wells each, numbered from 1 to 12.

The distribution of antigens is as follows :

- 100 µl of the viral antigen, diluted in carbonate buffer at pH 9.6, is deposited in each of the wells of rows marked ⊕

1 - 2 - 5 - 6 - 9 - 10

- 100 µl of the control antigen, diluted in carbonate buffer at pH 9.6, is deposited in each of the wells of rows marked ⊖

3 - 4 - 7 - 8 - 11 - 12.

The dilution of the viral antigen is titrated at each viral production. Several dilutions of viral antigen are tested and compared to positive and negative known controls (at several dilutions) and to human anti-IgG labelled with peroxydase, the latter being also tested at several dilutions.

As a rule, the proteic concentration of the preparation is of 5 to 2.5 µg/ml.

[redacted] concentration is used for the [redacted] antigen.

The plates are closed with a plastic lid and are incubated overnight at 4°C.

Then they are put once in distilled water and centrifuged. The wells are then filled with 300 µl of non foetal calf serum at 20 % in PBS buffer.

The incubation lasts 2 hours at 37°C (covered plates).

The plates are washed 3 times in PBS buffer with TWEEN 20, 0.05 % (PBS-tw buffer) :

- first washing 300 µl
- second and third washing 200 µl/well

The plates are carefully dried and sealed with an adhesive plastic film. They can be stored at -80°C.

ELISA reaction : antibody titer assay.

After defreezing, the plates are washed 3 times in PBS-TWEEN. They are carefully dried.

The positive and negative control sera as well as the tested sera are diluted first in the tube, with PBS-TWEEN containing 0.5 % bovine albumin.

The chosen dilution is 1/40.

- 100 µl of each serum are deposited in duplicate on the viral antigen and in duplicate on the control antigen.
- The same is carried out for the positive and negative diluted sera.
- 100 µl of PBS + TWEEN + bovine serum albumin are introduced in two wells ⊕ and in two wells ⊖ to form the conjugated controls.

The plates equipped with their lids are incubated for 1 h 30 at 37°C.

They are washed 4 times in PBS + TWEEN 0.05 %.

- 100 µl of human anti-IgG (labelled with peroxydase) at the chosen dilution are deposited in each well and incubated at 37°C.

[redacted] buffer. They are carefully dried.

Revealing the enzymatic reaction is carried out by means of a orthophenylene-diamine substrate (0.05 % in citrate buffer pH 5.6 containing 0.03 % of H_2O_2).

100 µl of substrate is distributed in each well.

The plates are left in a dark room for 20 minutes at the laboratory temperature.

Reading is carried out on a spectrophotometer (for microplates) at 492 nm.

Sera deemed as containing antibodies against the virus are those which give a ODD (optical density difference = optical density of viral antigen less optical density of control antigen) equal to or higher than 0.30.

This technique enables a qualitative titration as well as a quantitative one. For this purpose, it is possible either to use several dilutions of the serum to be assayed, or to compare a dilution of the serum with a range of controls tested under the same conditions.

The table hereafter provides first-results of serological investigations for LAV antibodies, carried out by using the above exemplified ELISA essay.

	Total examined	ELISA-LAV		ELISA-HTLV1 (Biotech)**	
		positive	%positive	positive	%positive
Lymphadenopathy patients*	35	22	(63)	5***	(14)
Healthy homosexuals	40	7	(17)	1	(3)
Control population	54	1	(1,9)	0	(<2,6)

* 28 homosexuals
3 Haitians (1 woman)
4 toxicomans (2 women)

** The number of positive sera is probably overestimated in this test, since no control of unspecific binding could be done.

*** Out of the 5 LAS HTLV1 positive, 3 were born in Haiti, 1 had stayed for a long time in Haiti and 1 had made several travels to USA.
All of them had also antibodies against LAV.

The table shows clearly high prevalence of LAV antibodies in the homosexual patients with LAS, the very low incidence in the normal population and also a moderate spread of virus infection in still healthy homosexuals. In the latter group, all the positive individuals had a high number of partners (> 50 per year). The incidence of HTLV antibodies was very low in all three groups (determined by using a commercial

[redacted] approximatively 20 % had LAV antibodies, but some of the sera were taken at a very late stage of the disease, with a possible negativation of the humoral response.

It should further be mentioned that lymphocytes of LAS patients do not produce detectable amounts of LAV-type virus. Particularly cells of lymph nodes from 6 more LAS patients were put in culture and tested for virus production, as described for patient 1. No virus release could be detected by RT activity. However, a p25 protein recognized by the serum of the first patient could be detected in cytoplasmic extracts of the T-cells labelled with ^{35}S-methionine in 3 other cases. This suggests partial expression of a similar virus in such cases. Moreover, all (6/6) of these patients had antibodies against LAV p25 proteins, indicating that they all had been infected with a similar or identical virus.

Interestingly, in lymphocytes of one of the patients (patient 2), there was a weak but definite immunoprecipitation of a band of similar size (p24-p25) with goat antiserum raised against HTLVI. Similarly, the patient's serum had antibodies against both HTLV and LAV, suggesting a double infection by either virus. Such cases seem rather infrequent.

The invention finally also relates to the biological reagents that can be formed by the LAV extracts containing the p25 protein or by the purified p25 protein, particularly for the production of antibodies directed against p25 in animals or of monoclonal antibodies. These antibodies are liable to form useful tools in the further study of antigenic determinants of LAV viruses or LAV-related viruses.

[illegible] with respect to the designation of some sub-sets of lymphocytes or related monoclonal antibodies, for ease of language, should in no way be opposed to the validity of any corresponding trademark, whether registered or not by its owner.

It should further be mentionned that the viral extracts, particularly viral lysates or enriched fractions can also be defined by reference to their immunological relationship or similitude with the extracts or enriched fractions containing a p25 protein as obtainable from the strain LAV1, IDAV1 or IDAV2. Thus any protein fraction which is capable of giving similar patterns of immunological reaction as do the protein extracts of LAV1, IDAV1 or IDAV2 with the same sera, must be considered as equivalent thereof and, accordingly, be deemed as encompassed by the present invention. A similar conclusion extends of course to the diagnostic means (process and kits) which may make use of such equivalent protein extracts.

The LAV1 virus has been deposited at the "Collection Nationale des Cultures de Micro-organismes" (C.N.C.M.) under n° I-232 on July 15, 1983 and IDAV1 and IDAV.2 viruses have been deposited at the C.N.C.M. on September 15, 1983 under n° I-240 and I-241, respectively. The invention encompasses as well the extracts of mutants or variants of the above deposited strains as long as they possess substantially the same immunological properties.

1.- Retrovirus extract immunologically recognized by sera of LAS and AIDS afflicted patients and obtained from a T-lymphotropic-retrovirus, whose preferential target consists of Leu 3 cells, which has reverse transcriptase activity requiring the presence of Mg^{2+} ions and displaying a strong affinity for poly(adenylate-oligodeoxy-thymidylase) [poly(A)-oligo$(dT)_{12-18}$], which has a density of 1.16 in a sucrose gradient, which has a mean diameter of 139 nanometre and a core of mean diameter of 41 nanometre, whose envelope proteins are not detected immunologically by the sera of LAS and AIDS afflicted patients, which is recognized immunologically by said sera and which contains a p25 protein which is not recognized immunologically by p24 protein of HTLV virus, which is free of a p19 protein.

2 - Retrovirus extract immunologically recognized by sera of LAS and AIDS afflicted patients and obtainable from any of the retrovirus deposited at the C.N.C.M. under n° I-232, I-240 and I-241.

3 - A retroviral extract which is immunologically related to the retroviral extracts of claims 1 or 2.

4 - The retroviral extract of any of claims 1 to 3 which consists of the crude lysate of said retrovirus.

5 - The p25 protein of the extracts of any of claims 1 to 4 which is in the purified state.

6 - A method for the in vitro diagnosis of LAS or AIDS, which comprises contacting a serum or other biological medium obtained from the patient to be diagnosed with the retrovirus extract of any of claims 1 to 4 or with the purified p25 protein of claim 5 and detecting the immunological reaction.

7 - A kit for assay of sera from LAS or AIDS afflicted patients which comprises :

- retroviral extract according to any of claims 1 to 4 or the purified protein of claim 5, which are labelled ;

- a lymphocyte extract obtained from a healty person ;
- buffers and, if appropriate, substrates for the vizualisation of the label ;
- means to detect the labelled conjugate resulting from the immunological reaction between the labelled reagent and the assayed serum.

8 - A kit for AIDS or LAS assay which comprises :
- a retroviral extract according to any of claims 1 to 4 or the purified protein of claim 5 ;
- labelled human anti-immunoglobulins ;
- a lymphocyte extract obtained from a healty person ;
- buffers and, if appropriate, substrate for the vizualisation of the label ;
- means to detect the labelled conjugate resulting from the immunological reaction between the labelled reagent and the assayed serum.

Antigens, means and method for the diagnosis of lymphadenopathy and acquired immune deficiency syndrome

The invention concerns a retrovirus extract containing a p25 protein which recognizes immunologically sera of patients afflicted with lymphadenopathy syndrom (LAS) or acquired immune deficiency syndrom (AIDS). It relates to a method and kit for *in vivo* assay of LAS or AIDS involving contacting sera from patients to be diagnosed for such diseases with said retrovirus extract and by detecting the immunological reaction, if any.

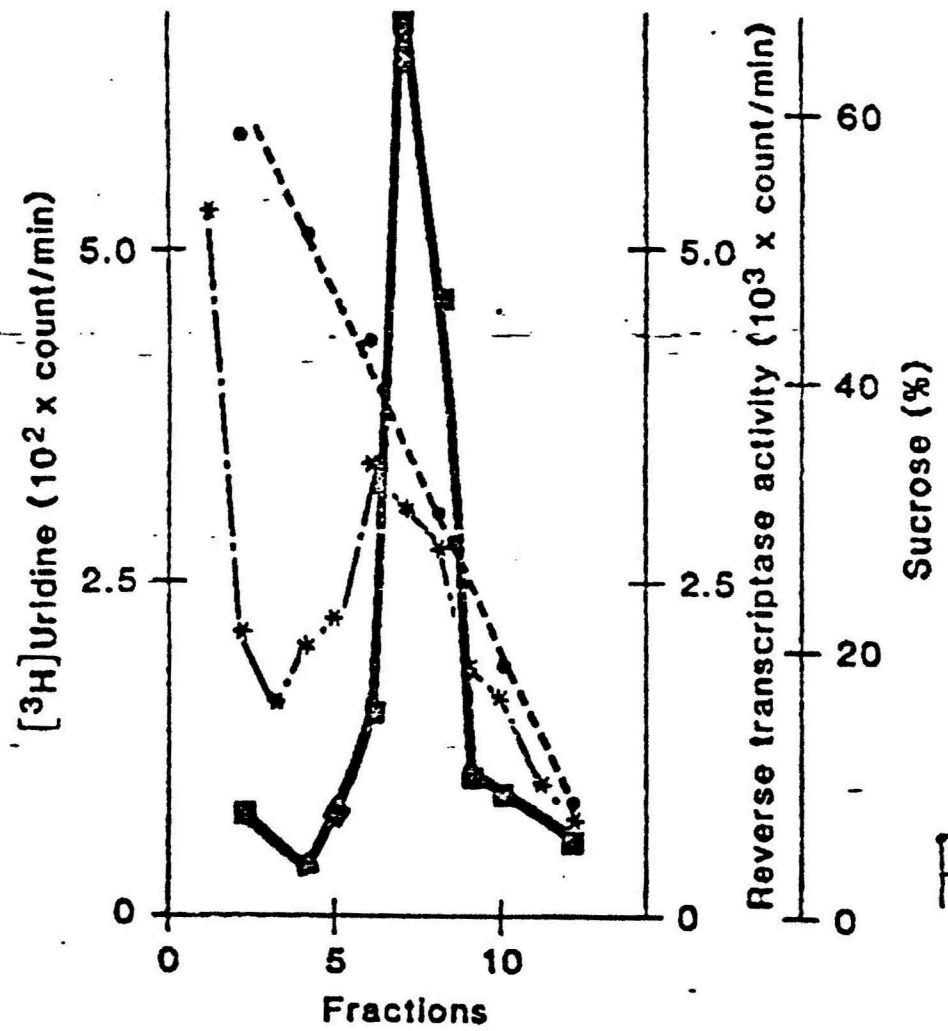

FIG 1

——— measure of reverse transcriptase activity on successive fractions of sucrose gradient.

—·—·— measure of acido precipitable material labelled with [^{3}H] uridine.

– – – – – density variation of the gradient.

NOT TO BE AMENDED.