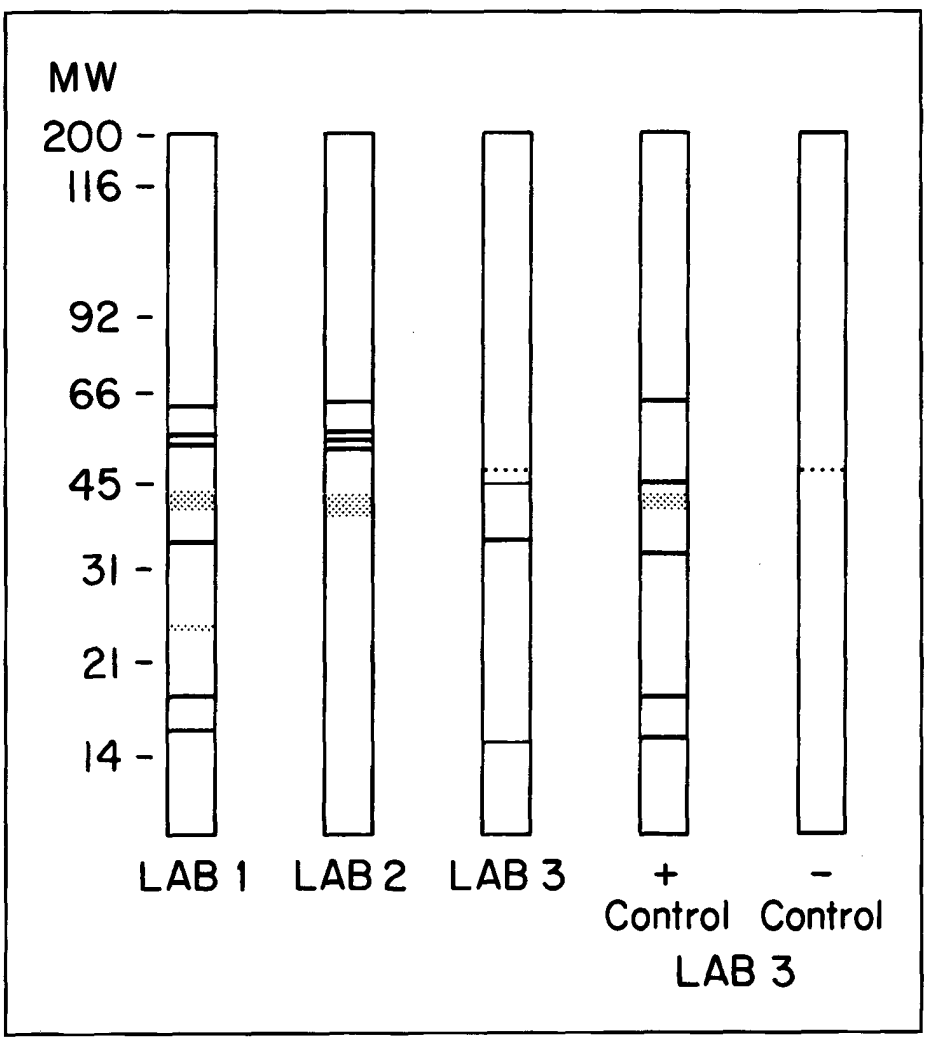
Letters to the Editor

A Patient with AIDS and a False Negative HIV Western Blot

To the Editor:

The diagnosis of HIV-related diseases in patients not recognized to be in high-risk groups has been facilitated by the availability of ELISA and Western blot assays. We report a case that illustrates potential difficulties involved with available assays.

A 37-year-old woman with cryptogenic cirrhosis developed unexplained diarrhea, progressive weight loss, fevers, thrombocytopenia, mild lymphopenia, and a 0.5 cm perirectal ulceration that increased in size to 14 cm over a 9-month period. Type 2 herpes simplex was cultured from biopsy of this lesion, and the ulcer healed with 6 weeks of acyclovir therapy. Her course was then complicated by *Mycobacterium avium-intracellulare* bacteremia, and cytomegalovirus retinitis which responded clinically to therapy with ganciclovir (BW 759U Burroughs Wellcome Inc., Research Triangle Park, NC). The patient denied intravenous drug use or transfusion prior to developing immunodeficiency. Over the previous 8 years, she had had four sexual partners, one of whom had moved to North Carolina from New York City 2 years previously but was unavailable for serologic testing. Her physical exam



Comparison of HIV Western blot analysis on a single serum specimen obtained from an AIDS patient late in her disease. Protein bands, possible HIV in origin, are represented by the solid lines (p17, p31, p53, p55, p64). HIV-gp41 and p24 are shaded, and nonspecific bands are depicted by the dotted line.

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was remarkable for bilateral axillary adenopathy, marked wasting, xerosis, and firm, nonnodular hepatomegaly (18 cm span). Lymphocyte characterization revealed 700 absolute lymphocytes per mm^3 with a helper/suppressor T cell ratio of 0.1%. She was anergic. Serologic testing revealed an HIV ELISA value of 1.7 (positive test $\geq$ 1.3: Abbott Laboratories, Chicago, IL), however, Western blot analysis was interpreted as negative for p24 or gp41 on two occasions (see Figure, lab 3).

Because of the relationship between false positive ELISA testing and liver disease,[1] studies on a single serum were repeated by lab 3 and by two independent laboratories. HIV ELISAs were positive at each lab. Serum was tested by an unlicensed ELISA procedure that controls for nonspecific reactions with H9 cellular material (H9 exclusionary test, ENI).[1] Little reactivity was noted (HIV, 1.714; H9, 0.058), suggesting a true positive HIV ELISA. Indirect immunofluorescence was positive in both lab 1 and 2,[1,2] although nonspecific immunofluorescence was noted by lab 1 at low serum dilutions. Western blot assays revealed differences in both the number and intensity of bands observed by each lab, with resultant differences in the final interpretation. Lab 1 and 2 identified the sample as positive, based on the detection of gp41. Lab 1 also detected a faint p24 band. However, lab 3 interpreted the sample as negative.

These data illustrate that Western blot results are dependent on a number of variables. HIV antigen source, blocking buffers, and antigen detection methods used in current assays are not standardized. For example, the three assays represented in the figure used three different antigen sources and two antibody detection methods. Additionally, the stage of illness of the patient is also correlated with variations in Western blot banding patterns.[3] This patient with AIDS presented atypically with cholestatic hepatitis and cirrhosis. Until the H9 exclusionary test, immunofluorescence and additional Western blot data were available, physicians caring for the patient were reluctant to inform her of her likely prognosis and infectivity. Nevertheless, blood and secretion precautions were instituted. Our experience suggests that when patients develop clinical and laboratory findings suggestive of AIDS, the possibility of a false negative or false positive ELISA and Western blot test must be considered. The three Western blot assays in the figure not only illustrate the variations in test results that may be encountered by different laboratories, but also point for the need for improved confirmatory tests for HIV.

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Failure of Sterilization Process Indicators

To the Editor:

We wish to draw your readers' attention to a sterilizer operation incident that could be prevented by relatively simple engineering modification as well as by attention to process quality control.

The "hi-lo" steam pressure valve was changed to its "hi" setting on an AMSCO Eagle 2053 Vacamatic Sterilizer in preparation for 270°F cycles, but the temperature control was inadvertently left set at 250°F following gravity sterilization cycles. This 250° setting will affect chamber conditions during the sterilization phase of cycles, but not jacket or conditioning phase chamber temperatures. Time-temperature graph (Figure) indicates achievement of 270° for a brief time during conditioning phase of subsequent cycles, followed by return to 250°, as set, for the balance of exposure time. The short exposure time is suitable for 270°, not 250°. This flaw was overlooked by the sterilizer operator, but later detected on routine review by a department supervisor. After these graphs were examined, sterilization process indicators (VAC—Diack Inc.) in recalled packs were also examined and found melted. A weekly biological indicator (Spordi—AMSCO) test had also been run with the first of the implicated lots, and was reported as no growth during 7 days' incubation.

Subsequent recall did not retrieve all packs prior to use in surgery. One case was delayed while sets were changed; three other cases received linen packs or instrument sets from the improper cycle. Revision of central sterilizing department procedures to prevent recurrence involved requiring two signatures to release each sterilizer load, and modifying a sterilizer to provide an interlock preventing operation if settings for cycle temperature and the "hi-lo" steam pressure setting are inconsistent.

Sterilization was attained, as indicated by the biological indicator strips, in spite of improper control settings and the possibility of superheated steam. Two hundred fifty degree steam might have been superheated by jacket heat to maintain 270° (the peak temperature recorded by thermocouple and VAC) at the top of the chamber. Alternatively, time at higher temperature during the conditioning phase alone may have achieved sterilization. The former prospect is consistent with Savage's[1] findings, and either case underscores a wide safety margin in the time-temperature integrators and overkill sterilization cycles used by hospitals.

We investigated a possibility that higher temperatures achieved during the conditioning phase of the cycle were responsible for spore strip sterilization, not expecting that this exposure alone would achieve the 2