

## Length of Incubation Time for Human Immunodeficiency Virus Cultures

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**Qualitative human immunodeficiency virus culture is a slow, labor-intensive, and expensive procedure, yet critical for the diagnosis of infants born to human immunodeficiency virus-seropositive mothers. We report that the cultures can be terminated at day 21 with minimal false-negative results but with considerable savings in both time and money.**

Human immunodeficiency virus (HIV) culture is expensive, labor-intensive, and slow. It is used primarily to determine whether an infant born to an HIV-seropositive mother is infected and to provide a viral isolate from known infected individuals for subsequent phenotypic testing (e.g., serum neutralization with the autologous isolate or drug sensitivity).

The consensus qualitative HIV flask culture used by the AIDS Clinical Trials Group (ACTG) has been described elsewhere (2, 3). Briefly,  $5 \times 10^6$  to  $10 \times 10^6$  peripheral blood mononuclear cells from the patient are isolated on Ficoll-Hypaque gradients, washed, counted, and cultured with an equal number of 1- to 4-day-old phytohemagglutinin-stimulated peripheral blood mononuclear cells from seronegative donors. The cultures are tested twice a week for the presence of p24 antigen by using commercially available enzyme immunoassays and are fed once a week with freshly stimulated donor peripheral blood mononuclear cells and once with fresh medium. Cultures are considered positive if they have had at least two consecutive supernatants with  $\geq 30$  pg of HIV p24 antigen per ml and when the antigen concentration is steadily increasing or is out of range of the enzyme immunoassay standard curve. A negative culture must have  $< 30$  pg of HIV p24 antigen per ml at all time points. An indeterminate culture is one that fails to fulfill the criteria of either being positive or being negative. Usually, these were cultures which had only a single supernatant with  $> 30$  pg of antigen per ml or ones that had several supernatants with consistently low concentrations of HIV antigen. The protocol dictates that negative cultures be kept for 28 days before being designated negative. If the culture supernatant tested at day 28 is positive, it should be kept in culture longer to allow additional aliquots to be tested. A recent review of the data suggests that these cultures can be terminated at day 21 with minimal false-negative results but with considerable savings in both time and money for the laboratory.

Data obtained from qualitative cultures at the University of North Carolina at Chapel Hill and the University of California at San Diego are shown in Fig. 1. Of 682 positive qualitative cultures, about 80% became positive during the first week of culture and 95% were positive by day 14. During the third

week the laboratories were able to detect an additional 4% for a total of 99% of the positive cultures. Only 7 of the 682 cultures which ultimately were judged to be positive were not detected by day 21 (false negatives). At the same time there were at least 353 negative cultures performed in the two labs which also had to be continued for the additional 7 days.

To determine whether similar results were being obtained in other ACTG laboratories, data from three ACTG protocols were analyzed (Table 1). ACTG 241 is a study of the safety and efficacy of the combination zidovudine-didanosine-nevirapine versus the combination zidovudine-didanosine in adult patients with CD4 counts  $\leq 350/\text{mm}^3$  and  $\geq 6$  months of nucleoside therapy. Of 268 cultures from this study, 237 were positive at both day 21 and day 28, 24 were negative at day 21 and day 28, and 6 were indeterminate on both days. There was only one culture of 268 which was negative on day 21 but which became positive by day 28 (a false negative).

ACTG 076 studied the effect of zidovudine during late pregnancy, labor, and delivery and during the first 6 weeks of life on the transmission of HIV to the neonate. Here, 791 of 803 cultures had the same result on day 21 as on day 28. An additional culture supernatant was indeterminate on day 21 and, according to the ACTG consensus protocol, would have been kept in culture until it became clearly positive. Ten cultures had clear positive or negative results on day 21 but became indeterminate by day 28. There was only one culture of 803 that was negative on day 21 which became positive by day 28.

ACTG 128 was a study comparing high-dose versus low-dose zidovudine on mildly symptomatic, HIV-infected children between the ages of 3 months and 6 years. In ACTG 128, 1,353 of 1,384 cultures had the same result on day 21 as on day 28. Here, 13 specimens would have been kept in culture a little longer because they were indeterminate on day 21. Thirteen cultures had clear positive or negative results on day 21 and became indeterminate by day 28. There were only 5 cultures of 1,384 which were negative on day 21 and which became positive by day 28.

In all, 7 (0.29%) of a total of 2,455 cultures would have been missed by terminating the cultures at 21 days instead of at 28 days of culture. Even terminating qualitative cultures on day 14 had a minimal impact on the overall results. When the culture results from ACTG 128 were analyzed at day 14, 1,279 cultures had the same result as on day 28 (93.7%). There were 42 specimens that would have been cultured longer because they

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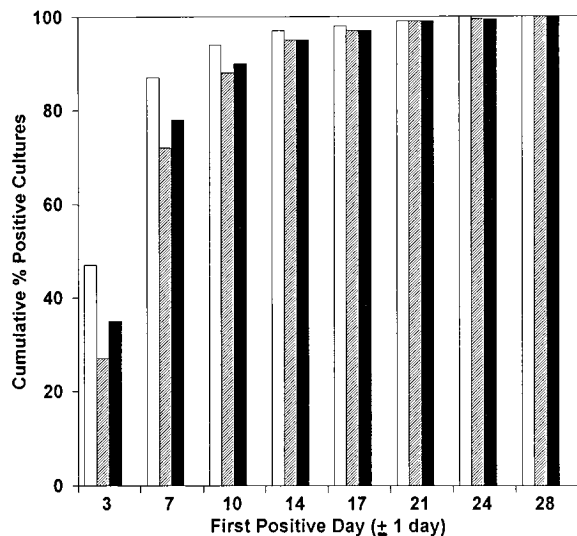


FIG. 1. Cumulative percent positive cultures by day in culture for 682 positive qualitative HIV cultures performed at the University of North Carolina (open bars) and the University of California at San Diego (shaded bars). Solid bars, combined data.

were indeterminate. There were 13 cultures with definite positive or negative results at day 14 which became indeterminate by day 28. There were only 23 false negatives (1.7%).

In addition, holding cultures for 35, 42, or even 60 days does not result in the detection of any more positive cultures (1, 4). Thus, even for the purpose of obtaining an isolate for resistance or other phenotypic testing, it is not beneficial to keep cultures for prolonged periods.

After the initial setup costs of a culture, we estimate that it costs approximately \$30 to 50 each week to continue to feed the culture with freshly stimulated peripheral blood mononuclear cells, harvest an aliquot, and test for the presence of p24 antigen. Considerable laboratory savings will be realized by terminating these cultures a week earlier.

On the basis of these results, the Virology Committee of the ACTG has recently approved a modification of the consensus

TABLE 1. Qualitative HIV culture results on day 21 and 28 of culture

Result <sup>a</sup>		No. of cultures <sup>b</sup>			
Day 21	Day 28	ACTG 241 (268)	ACTG 076 (803)	ACTG 128 (1,384)	Total (2,455)
+	+	237	91	1,106	1,434
-	-	24	685	162	871
?	?	6	15	85	106
-	+	1	1	5	7
?	+	0	1	13	14
-	?	0	9	6	15
+	?	0	1	7	8

<sup>a</sup> +, positive; -, negative; ?, indeterminate.

<sup>b</sup> The totals are given in parentheses.

protocol. Cultures should be terminated after day 21, unless a positive result is found earlier.

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