Practical Quantitative Assay for 5-Fluorocytosine in Serum and Other Body Fluids

ROBERT G. BLAKER AND BARBARA J. DOUTT
Bio-Science Laboratories, Van Nuys, California 91405

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A method is described for assaying 5-fluorocytosine levels in serum or other body fluids. It is simpler and less expensive than the standard cylinder plate assay and is therefore more practical for routine diagnostic laboratory testing.

5-Fluorocytosine (5-FC), studied for several years as a possible antifungal therapeutic agent, is now generally available as Ancobon (Hoffman-LaRoche, Inc.) and is intended primarily for the treatment of serious Candida albicans and Cryptococcus neoformans infections (1, 4). The frequent monitoring of serum antibiotic levels is recommended, especially in patients with current or potential renal insufficiency. The cylinder plate assay described by Shadomy (3) is an excellent method, but somewhat cumbersome and expensive. Below is described a method we have devised which has proved to be relatively simple and inexpensive for use in the routine diagnostic laboratory and which is accurate to ± 20%. In essence, we have adapted Sabath's disc diffusion method (2) for quantitating aminoglycoside antibiotics to a yeast-5-FC system. All serum specimens used by us in this study were from patients with excessively high serum levels related to varying degrees of renal dysfunction.

The test organism used was a strain of Saccharomyces cerevisiae which was isolated repeatedly from a chronic knee joint infection and maintained on Sabouraud agar; the minimal inhibitory concentration of 5-FC for this strain was 0.1 μg/ml as determined by the method of Shadomy (3). The yeast was grown in yeast nitrate base (YNB) broth (Difco 0392-15-9) for 24 hr at 37 C; 1.0 ml of the culture was added to 100 ml of YNB agar at 50 C, and 12.0 to 12.5 ml was immediately dispensed to each of several 150-mm petri dishes on a flat, level surface. Seeded plates are stable at 5 C for 5 to 7 days and were packaged in plastic bags to retard drying.

Filter paper discs, 0.25 inch in diameter (Schleicher and Schuell, no. 740-E), were loaded with 20 microliters each of standard solutions of 5-FC diluted in normal human serum (NHS) to concentrations of 2, 4, and 6 μg/ml, one pair of discs per standard, and one set of standards per plate. Patient sera were diluted 1:10 in NHS for screening, in duplicate. Loaded discs were evenly distributed on seeded plates with one of each pair of discs 180° from the other to compensate for slight differences in agar thickness, and incubated inverted at 37 C for 18 to 24 hours. Up to four specimens could be tested on each plate.

For each plate, a graph was prepared on semilog paper: the logarithmic axis was used for antibiotic concentration, and the arithmetic axis for average diameter, in millimeters of the zones of inhibition observed. The average diameters of inhibition zones for patient sera were read off the graph and multiplied by the dilution factor; if zones are smaller than the 2 μg standard or larger than the 6 μg standard, the test should be repeated with more concentrated or more dilute specimen, respectively. Figure 1 illustrates why each plate needs its own set of standards; the standard curve for each of three plates was plotted.

![Figure 1](https://example.com/figure1.png)

**FIG. 1. Plots of 5-fluorocytosine (5-FC) standards on three separate plates of seeded yeast nitrate base agar. The average diameters of zones of inhibition are plotted for 2, 4, and 6 μg/ml of 5-FC on a semilogarithmic scale to illustrate plate-to-plate variation.**
The reproducibility of the test system was studied by using 12 sera from patients with excessively high serum levels of 5-FC, diluted 1:20 in NHS and tested on seven separate occasions with four separate lots of seeded YNB agar plates. (When more reasonable serum levels are anticipated, e.g., 5 to 30 micrograms per milliliter, serum dilutions of 1:5 or 1:10 are more appropriate.) Reproducibility was generally within ± 20%, more than satisfactory for clinical use, and obviously was better than the ± 50% reproducibility associated with tube dilution methods.

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LITERATURE CITED