

CHEMILUMINESCENCE RESPONSE IN NORMAL HUMAN PHAGOCYTES. I. AUTOMATED MEASUREMENTS USING A STANDARD LIQUID SCINTILLATION COUNTER

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SUMMARY We present a fast, simple and highly accurate method for measuring Phagocyte Chemiluminescence by using a standard liquid scintillation counter with a simple modification. The method allows the measurement of multiple samples automatically, with standardized mixing and measurement times, at a constant temperature. This is achieved with a minimum of technician time as opposed to the single measurement facility of many of the available manual luminescence photometers. The modifications can be made to any standard liquid scintillation counter, are simple and inexpensive, can allow the handling of large numbers of samples in a short time and improve standardization.

INTRODUCTION

EVIDENCE exists both *in vivo* and *in vitro* that chemiluminescence (CL) and microbial killing reflect similar events (1-3). The use of CL measurements in the determination of phagocytic and opsonic defects has been shown to have several advantages over conventional methods (4-7). One of these is the short time required to obtain results. In contrast to *S. aureus* killing (24-48 hr) (8) or to radio-isotopic methods (6-24 hr) (9-11), CL results can be obtained in as little as one hour after cell isolation. The advantage of using an LSC over a photometer in recording CL results is that only single measurements can be made on a photometer, whereas the LSC can be automated in order to reduce operator time and facilitate the measurement of multiple samples. Because most laboratories have the use of an LSC, we present a method for determining CL response of phagocytes utilizing a simple modification to any standard LSC which provides a system superior to any photometer presently available. Advantages are increased sample number throughput, requiring less technician time but maintaining absolutely reproducible testing conditions.

MATERIALS AND METHODS

Phagocytic Cells

Human neutrophils (PMN) or monocytes (MN) were obtained by taking 10-20 ml of heparinized blood (10 IU/ml) and utilizing ficoll-paque separation techniques (12).

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Zymosan

Zymosan (Sigma) was prepared by boiling 500 mg in 50 ml phosphate buffered saline (PBS) washing and resuspending to 50 mg/ml in PBS. Zymosan was opsonized for 30 minutes with appropriate serum samples.

Assay Procedure

Standard 25 mm scintillation vials were used for each sample. 2.2 ml PBS, 1 ml cell suspension (1×10^6 phagocytic cells in Hanks' Balanced Salt Solution), 700 μ l serum (patient or control) and 100 μ l Luminol were added to vials which were placed in the LSC to obtain background counts. After 15-20 minutes, 1 ml Zymosan (5 mg) was added to each vial and CL results recorded automatically for 30-60 minutes. Samples were moved into the detector area, counted for 6 seconds followed by a 12 second interval before the second sample was counted. Counting time can be varied as required.

Measurement Device

Automatic repeat counting of samples was achieved through a simple modification of a Packard 2660 LSC (Packard Instrument Co.) with modification provided by Packard. The modification involves installation of two additional integrated circuits and associated components in the sample changer control logic along with a switch to select the Rack Repeat function. This modification caused the sample changer mechanism to return to vial No. 1 immediately after counting vial No. 10. Since most LSC's utilize either a belt or rack system any standard LSC can be used and modified accordingly. The modifications involve either a reversal of the belt or disengaging of the transport mechanism. In the Packard 2660 up to 10 samples loaded in the rack may be sequentially repeat counted when this function is selected.

Assessment of Results

Because of the automatic changes in the LSC used in this assay, the time between each sample measurement remains constant but can be varied to suit the experiment. Therefore, by measuring elapsed time from the start of the assay, a continuous temporal trace of luminescence is possible (fig. 1). By connection of a computer/plotter to the output device of the LSC, a complete analysis of peak height, slope and total counts can be provided, complete with graphs, within 10 minutes of the last sample measurement.

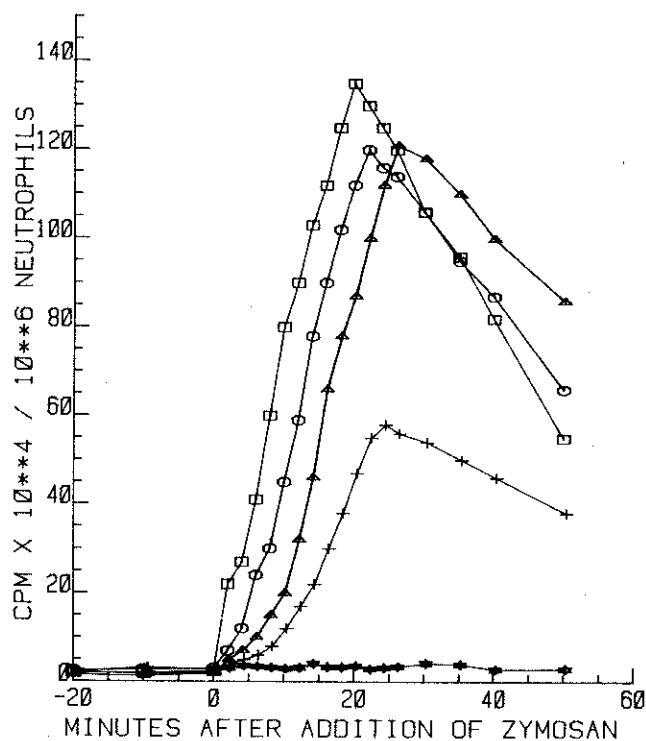


Fig. 1. Computer drawn curves from a familial study. Controls \square , \circ ; Father \triangle ; Mother \circ ; Child with CGD \bullet ; Slope, area under curve and peak height are computed from each curve.

DISCUSSION

We present a viable alternative for the assessment of phagocytic function of PMN or MN utilizing a standard LSC which is available in most clinical laboratories. The modifications necessary are cheap and relatively simple and provide a more suitable system for the routine assessment of phagocytic function than any of the readily available photometers. These instruments provide only a single vial measurement and are not ideally suited to the multi-sample handling. Most photometers do not provide a mechanism to mix the vial contents. If multiple samples are to be measured they each have to be manually placed into the sample chamber for each measurement. This involves continuous handling by the technician and careful timing in order to measure each sample for the same time interval. Problems also arise in that samples cannot be treated in exactly the same manner. Even though some available photometers have multiple tube capacity, they have no advantage in saving of technician time nor in the accurate replication of results.

By utilizing our methods in performing CL estimations, over 50 individual cell or serum samples each resulting in a temporal CL trace takes no more than 3 hours. Each CL trace is performed over a 30–40 minute duration with approximately 15 individual CL

measurements over this time. The LSC output has been connected directly to a Hewlett-Packard 85 desktop computer which analyses each individual trace and provides a CL result in terms of several parameters as well as a hard copy of each curve.

By using the modified LSC, 10 different samples can be tested simultaneously. Each of the 10 vials is moved into the detector bay at 12 second intervals and counted for 6 seconds or any required time. After each of the 10 vials have been counted they return to be counted over again. The movement of the sample rack provides continuous standardized mixing of each vial. Thus in 30–40 minutes a complete analysis of 10 samples can be achieved accurately and in a standardized manner. More racks of up to 10 samples can be repeat counted in a similar manner.

We have investigated a number of the available photometers and have found that they are unsuitable for testing of multiple samples. This is because each sample must be handled several times in order to achieve a temporal trace of luminescence. By using an LSC as described, a large number of samples can be tested automatically.

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