

LIQUID SCINTILLATION IN MEDICAL DIAGNOSIS

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With the tremendous increase in the application of radioassay, particularly radioimmunoassay, in the clinical laboratory liquid scintillation counting became an indispensable tool in diagnostic medicine. Few publications, however, have concerned themselves with problem areas which occur with the method in the clinical laboratory. The purpose of this presentation is to summarize our experiences with the liquid scintillation technique in the clinical situation.

The Digoxin Example

Before entering into a discussion on the application of radioassay in the clinical laboratory, it is necessary to inspect data from a typical radioassay. In order to appreciate the relative importance of a diagnosis in this area, we have selected the radioimmunoassay of digoxin as a particular example.

Digoxin is a common drug of the digitalis or cardiac glycoside group used to treat advanced heart disease in humans. Due to a number of patient variables the exact dosage of digoxin must be tailored to the individual and radioimmunoassay is used to monitor serum levels. Too low a dosage of digoxin renders the drug ineffective or non-therapeutic. Too high a dosage has been implicated in the deaths of patients due to severe toxicity above the therapeutic level (1-3). The dosage for each patient must be carefully adjusted to maintain serum levels in a very narrow therapeutic range of about 1-2 ng/ml.

Table 1 presents data obtained from a typical digoxin radioimmunoassay using a tritiated digoxin derivative. In order to achieve the requisite sensitivity for the assay a limited amount of tracer mass is added to each assay tube. Thus, the subsequent counting rate is quite low within the range of diagnostic significance. It is easy to see, therefore, that a small error in the counting rate will have a

significant effect on the ultimate diagnosis.

Note that the background counting rate is almost identical to the counting rate of the last sample. This means that a long counting time must be employed to ensure accurate measurement of both background and sample counting rates. Secondly, notice that the therapeutic range, that is between 1-2 ng/ml, comprises a narrow counting rate range of only ~200 cpm and a small error in measurement of the counting rate could result in a faulty diagnosis. Therefore, the effect of various sources of background or spurious counts becomes quite important in liquid scintillation digoxin radioimmunoassay.

Chemiluminescence

Many clinical users of the liquid scintillation technique are totally unaware of the possibility or consequences of chemiluminescence. To demonstrate the effect chemiluminescence can have on an assay, 300 μ l of a simulated non-radioactive digoxin radioimmunoassay sample were added to 3.0 ml of a Triton:toluene (1:2) scintillation cocktail (4). The samples were placed in a liquid scintillation counter and counted for one minute repeatedly for five hours. Even at 130 minutes after insertion of the sample into the liquid scintillation counter (Figure 1), the rate of chemiluminescence was still three times background. Since the assay for digoxin in many cases needs to be a stat procedure, one wonders whether the liquid scintillation technique should be used. Even in the best commercial liquid scintillation cocktails, which have reducing agents and acidic buffers to minimize chemiluminescence, the rate of spurious counts was still six times background at one hour after preparation of the sample.

Table 2 demonstrates that as expected the chemiluminescence counting rate increases dramatically as the serum sample size increases. Therefore, the smallest practical sample should be used.

It is imperative that samples be allowed to equilibrate preferably for four hours in the liquid scintillation counter prior to counting. The use of a chemiluminescence monitoring device, such as the Photon Monitor, is highly recommended in these cases. However, these devices are set at an arbitrary luminescence rate which may be unsatisfactory in certain assays. Users of this type of instrumental rejection of samples should consult their manual to be certain that the

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Table 1. Typical Tritiated Digoxin Radioimmunoassay¹

<u>Sample CPM</u>	<u>Net CPM</u>	<u>Digoxin Concentration ng/ml</u>
1245	1061	0 Non-therapeutic
1137	953	0.4
962	778	1.0 Therapeutic
746	562	2.0
628	444	3.0
502	318	5.0 Toxic
372	188	10.0

Background - 184 cpm

¹ Schwarz/Mann Digoxin Radioimmunoassay Kit brochure (undated).

Table 2.

The Effect of Sample Volume on Chemiluminescence Rate

Decreasing volumes of a non-radioactive serum were added to 3.0 ml cocktail in a mini-vial. The chemiluminescence rate was measured for one minute five minutes after mixing.

<u>Sample Volume (microliters)</u>	<u>Chemiluminescence Rate (Net cpm)</u>
500	4742
400	2110
300	1002
200	493
100	147

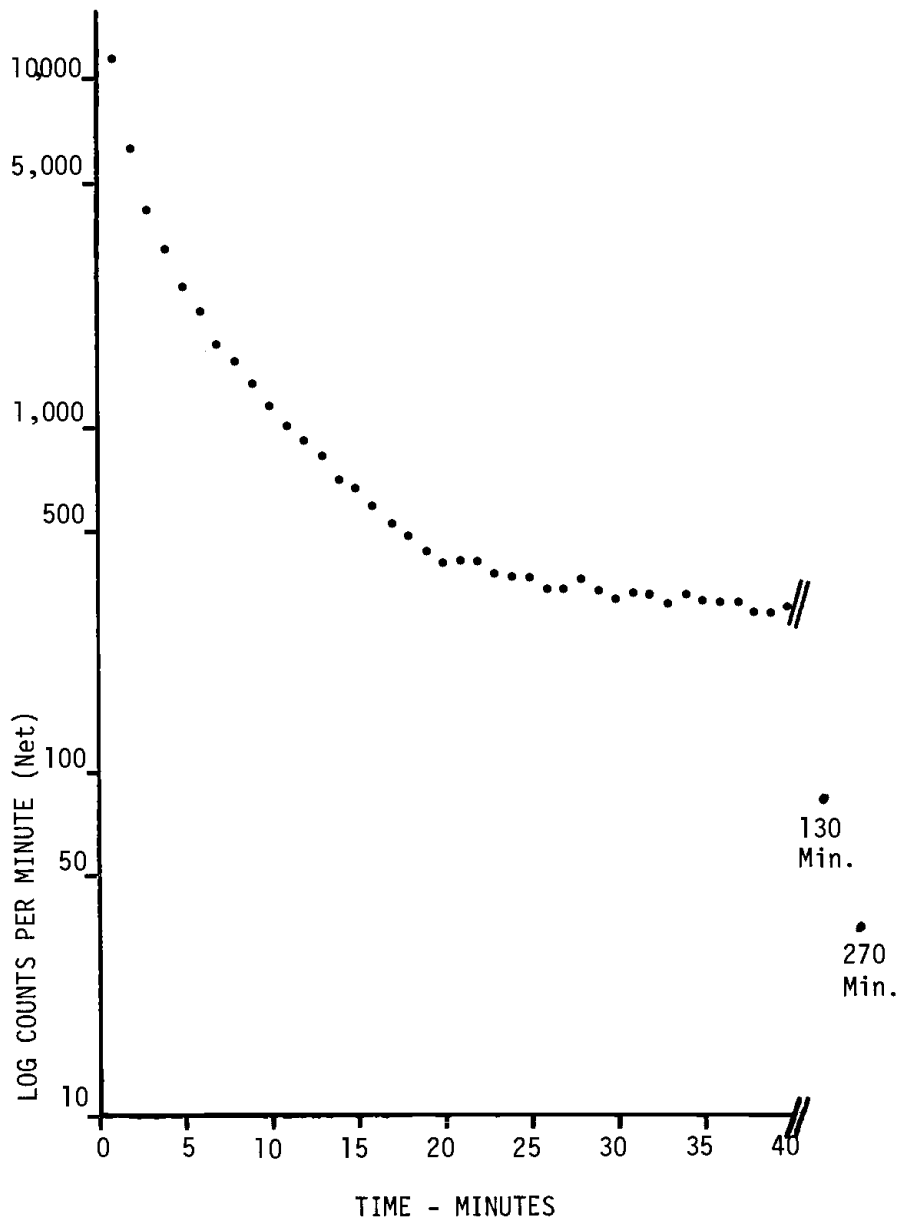


Figure 1 CHEMILUMINESCENCE OF RADIOIMMUNOASSAY SAMPLE

rejection rate is low enough for the particular assay in question.

Photoluminescence is caused by exposure of vials, caps and cocktail to direct sunlight or fluorescent lighting. Black caps are less prone to photoluminescence than white caps (5). Cocktail solution should be stored in amber bottles in the dark and should be allowed to stabilize for 24 hours after preparation. Direct sunlight and fluorescent lighting should be excluded from the counting laboratory.

Referring back to our digoxin example in Table 1, an undetected increase in background or chemiluminescence will result in a falsely low measure of digoxin. This may lead to the prescription of an increased doseage to the patient with the resultant risk of digitoxicosis.

Counter Performance

While the quality control of radioimmunoassays is usually quite rigid in terms of the characteristics of the assay calibration curve, one must also monitor the characteristics and shape of the quench correction curve. Figure 2 is a photograph of our counter quality control sheet which displays a problem occurring with one of our spectrometers when it began to experience phototube fatigue. One can see from the dates that over the period of one month the sample channels ratio calibration curve drifted considerably out of the range normally expected. This resulted in inaccurate quench correction factors for the assay. When the faulty phototube was replaced, the quench correction curve returned to normal and remained stable. It is recommended that the quench correction curve be monitored and recorded with each run as a means of measuring instrument performance.

Quench Correction

It is not at all uncommon for commercial radioimmunoassays or publications on radioimmunoassay to present data in cpm which has not been corrected for quenching. While most of us in this audience are well aware of the dangers in attempting to tabulate data which has not been corrected for quenching, it is common practice, I am afraid, in many clinical laboratories.

In order to demonstrate the effect of both chemical and color quenching of different sera, 8 human and 2 commercial control

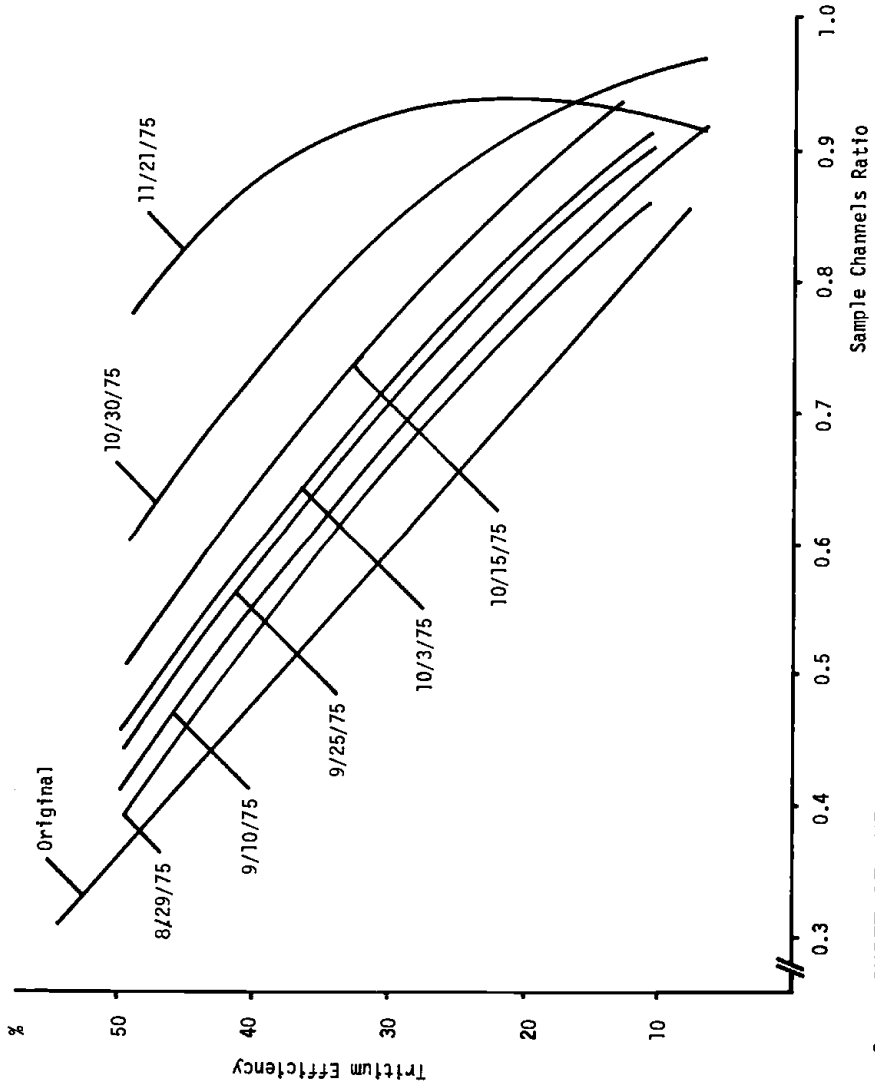


Figure 2 SHIFT OF QUENCH CORRECTION CURVE WITH TIME DUE TO INSTRUMENT MALFUNCTION

sera were analyzed by the manner described above. In this case, however, the liquid scintillation cocktail was spiked with $30,120 \text{ cpm} \pm 401 \text{ cpm}$ ($\bar{X} \pm \sigma$) tritiated toluene and all vials were precounted prior to the addition of serum. Samples were equilibrated 24 hours in the dark to eliminate chemiluminescence.

Table 3 indicates the loss in counts due to quenching of different sera. Based upon the typical digoxin counting data in Table 1 one can see that a significant error is introduced when quench correction is not employed. The range of counting rates in the samples with identical amounts of tritium was 3073-6058 cpm. It is also interesting to note that commercial control sera, which are usually charcoal stripped human sera, do not count with the same relative counting efficiency as patient sera.

Color quenching can be reduced and counting efficiency can be improved by using a smaller sample and by maximum dilution of sample in scintillation fluid. The use of 20 ml vials diluted to the very top helps to minimize the effect of color quenching. When highly colored sera are encountered in a digoxin assay, it is mandatory that internal standardization techniques be employed. Since it is well known that external or sample channels ratio methods of quench correction do not totally correct for color quenching, the counting rate of the sample will be grossly underestimated. In the case of the digoxin radioimmunoassay the patient's serum would appear to contain more digoxin than is actually present. Based upon the assay alone a physician might prescribe a decreased dosage of digoxin.

Contamination From In Vivo Scanning Agents

Although it is rare for a contaminated radioactive serum sample to come into the laboratory for a radioimmunoassay test, a number of documented cases have occurred and one must be prepared for this eventuality. One must ensure that the serum sample has not been taken from the patient following a nuclear medicine in vivo scanning test. Since these agents are invariably strong gamma emitting nuclides, they can be picked up rather readily in most cases by looking at an auxiliary channel which has a lower discriminator set beyond the tritium beta spectrum end point. A quick glance at the counting data from the auxiliary channel will reveal radioactively contaminated sera.

Table 3 Quenching Effect of Different Sera

Cocktail was pre-spiked and each vial pre-counted to $34,120 \pm 401$ dpm prior to the addition of sample.

<u>Human Sera</u>	<u>Net Counts per Minute</u>
A	4308
B	5100
C	3082
D	3565
E	5548
F	5130
G	3769
H	3073
<u>Commercial Control Sera</u>	
A	5957
B	6058

Sample Homogeneity

Many liquid scintillation cocktails will not adequately dissolve the strong buffers and large amounts of serum used in radioummunoassay. Samples should be inspected visually after the four hour equilibration period, particularly when refrigerated counters are used. A sample which is apparently homogeneous at ambient temperature may undergo phase separation at 8°C.

The usual way to monitor sample homogeneity is to utilize the double ratio test of Bush (6). This method involves the use of both sample channels ratio and external standard channels ratio to compute counting efficiency. A sample which results in grossly different efficiency determinations by both sample channels ratio and external standard channels ratio is nearly always heterogeneous. Several computer programs have been developed to test each sample by the double ratio method and to flag aberrant samples. A good reference on this topic is Glass (7).

Recommendations

Based upon our experience with clinical samples we offer the following suggestions which are intended to minimize spurious results.

1. Utilize 20 ml glass counting vials with black caps (5).
2. Utilize the minimum practical sample size.
3. Dilute the sample to the maximum capacity of the counting vial.
4. Add a reducing agent to the cocktail (8).
5. Maintain a pH < 7 in the cocktail (9).
6. Adopt the double-ratio test (6).
7. Monitor quench correction curves with each run.
8. Avoid exposure of samples to direct sunlight or fluorescent lighting.
9. Never open the counter lid while samples are counting.
10. Allow samples to equilibrate a minimum of four hours and inspect them prior to counting.
11. Monitor an auxiliary channel for radioactively contaminated samples.
12. Use a Photon Monitor or similar device to detect chemiluminescent samples.
13. For highly colored sera correct for quenching by internal standardization.

Summary

In summary one might conclude that because of the pitfalls which can occur in the liquid scintillation technique, the method has fallen from being a useful analytical tool to somewhat of an unpredictable, unreliable technique in the clinical laboratory. Radioimmunoassay users have gone to great lengths to circumvent liquid scintillation counting by labelling compounds with gamma or X-ray emitters, so that a "gamma" counter can be substituted. In that sense I believe we have failed the medical community by not devising novel, simple solutions to the problems described above. Hopefully, sessions such as these will lead to simplifications and improvements in liquid scintillation technology which will encourage, rather than discourage, its use as an analytical tool to solve problems in medicine and research.

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