

# STABILITY OF STORED SERUM BILIRUBIN IN CALABAR, A TROPICAL REGION: TIME, LIGHT AND TEMPERATURE EFFECT

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(Received 15 September 1999; Revision accepted 17 January 2000)

## ABSTRACT

The best storage time for and temperature-dependent photolability of bilirubin in sera were assessed. One hundred pooled icteric human sera were divided into four aliquots each, kept under different storage conditions, and analysed for bilirubin at designated intervals by Powel's method of Van der Bergh reaction. Percentage decrease of bilirubin with time was determined for each of the samples stored at 4°C and room temperature (as protected and unprotected from light). Storage at 4°C in the dark produced the least and insignificant lability for total bilirubin up to 3 hours of storage. At room temperature, unconjugated bilirubin was more labile than conjugated bilirubin, but the reverse was true at 4°C. Temperature-dependent photolability was evident as samples exposed to both light and higher temperature (25°C) showed significant decrease ( $p < 0.01$ ) in both bilirubin fractions at 15 minutes of storage while those exposed to light or higher temperature produced significant decrease at 30 minutes. It is concluded that serum bilirubin is best stored in the dark and at low temperature. A chart to correct for effects of time, light and temperature emanating from delay between blood sampling and analysis for bilirubin in Calabar and similar geographical locations is provided. Such correction would enhance reliance on bilirubin results in monitoring icteric patients.

**KEY WORDS:-** Bilirubin, Photolability, Correction-chart.

## INTRODUCTION

Measurement of bilirubin levels in sera is useful in management of hepatobiliary diseases (Doumas and Wu 1991). One recognised difficulty with bilirubin estimation is its photolability. Bilirubin undergoes photodegradation to some intermediate products upon exposure to white light, either artificial or sunlight (Blanckaert 1990, Tietz *et al* 1994). The photodegradation arises from either structural isomerization or photo-conversion (Doumas and Wu 1991), configurational/geometrical isomerization (Tietz *et al* 1994), and/or photo-oxidation (Blanckaert 1990). Photodegradation produces lower bilirubin values since the products are immeasurable by the van der Bergh reaction. There is controversy as to the susceptibility of bilirubin fractions to photodegradation (Cremer *et al* 1964, Doumas and Wu 1991). In addition, temperature-dependent photolability of bilirubin has been reported (Tietz *et al* 1994). A fall out from these problems is the inability to correlate bilirubin results especially when a patient's condition is monitored following treatment. Present study was designed to determine the effect

of light, temperature and time on stability of bilirubin in serum in Calabar, Nigeria, a tropical region with high humidity and high room temperature (25°C) - factors different from those in temperate regions where studies have been conducted. The aim was a derivation of a correction factor or chart for bilirubin loss at the end of study.

## MATERIALS AND METHODS:

Icteric sera from one hundred patients seen at University of Calabar Teaching Hospital, Calabar were pooled and dispensed into clean containers each aliquot enough for bilirubin determination, and divided into four sets. The pool was maintained in a dark container and dispensing was done with minimal exposure to light. Two of these sets of containers were light-protected by wrapping in black carbon paper. The other containers were exposed to light (unprotected). One container each of the light-protected and unprotected specimens was stored at 4°C and another at room temperature (25°C) away from direct sunrays. Bilirubin was determined at zero hour and at designated intervals up to 12 hours. Bilirubin determination was by method of Powel

(1944). The rate of decrease of bilirubin in the test settings was assessed and statistical significance was determined by use of chi-squared test.

**RESULTS:**

Storage of specimens in the dark (black carbon paper) reduced rate of bilirubin lability when compared to storage in light at both room temperature (25°C) and 4°C. P < O. 01: Table 1. This protection was evident even at 15 minutes of storage. Bilirubin was more stable at lower temperature (4°C) than at 25°C (P < 0.01). Fig. 1 shows a plot of percentage decrease of bilirubin against storage time.

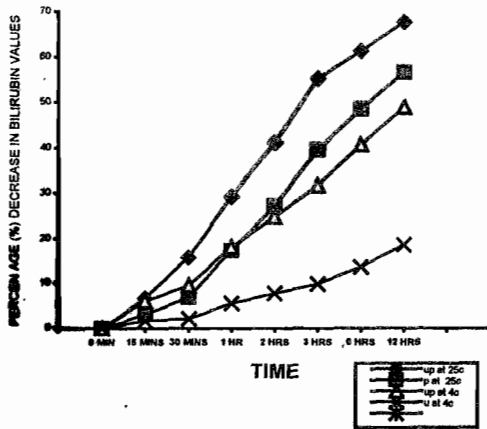


Figure 1: Percentage decrease in Bilirubin per time at different storage conditions.

Unconjugated bilirubin was more photolabile than conjugated bilirubin at 25°C and in specimens exposed at 4°C. Fig. 11. However, at 4°C in the dark, conjugated bilirubin was more susceptible to lability than unconjugated bilirubin. Fig. III.

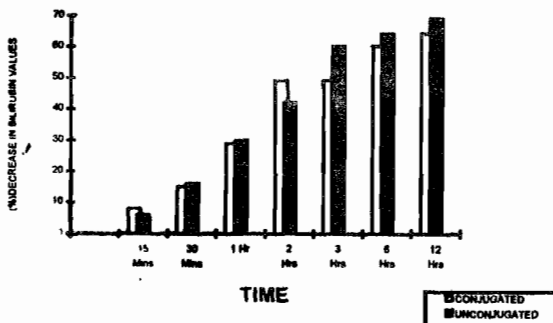


Figure II: Lability of Bilirubin fractions at 25°C (unprotected)

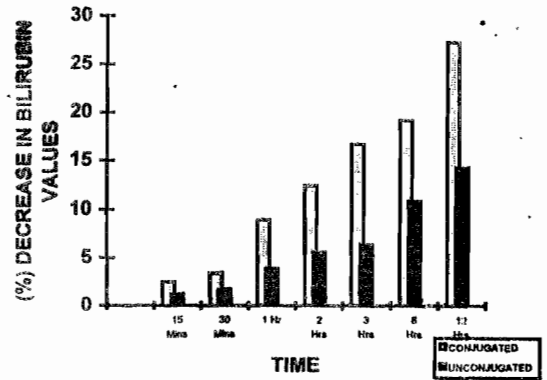


Figure III: Lability of Bilirubin fractions at 4°C (protected)

**DISCUSSION**

Bilirubin is known to be photolabile. In this study it has been observed that bilirubin photolability is not only reduced by storage away from light (in darkness) but also by low temperature. Storage at 4°C in the dark produced statistically stable levels of total bilirubin up to 3 hours. The finding of temperature-dependent photolability of bilirubin agrees with reports elsewhere (Doumas and Wu 1991, Tietz *et al* 1994) but a shorter time of bilirubin stability in the refrigerator (4°C) has been recorded in this study - 3 hours as against the 3 days reported by Tietz *et al* (1994). Most documented studies were conducted in temperate region where room temperature average 15°C. Calabar is a tropical region with average room temperature of 25°C. Geographical and climatic variations in experimental conditions such as this may explain the observed differences.

TABLE 1: DECREASE IN BILIRUBIN PER TIME AT DIFFERENT STORAGE CONDITIONS

TEST CONDITION OF SAMPLE	PERCENTAGE (%) DECREASE IN BILIRUBIN VALUES							
	0*	15	30	1	2	3	6	12
	MIN	MINS	MINS	HR	HRS	HRS	HRS	HRS
UP AT 25°C	0	6.7	15.8	29.2	41.2	55.2	61.3	67.7
P AT 25°C	0	3.1	6.9	17.3	27.1	39.6	48.5	56.6
UP AT 4°C	0	6.1	9.5	18.1	24.8	31.6	40.7	48.9
P AT 4°C	0	1.7	2.2	5.6	7.8	9.8	13.6	18.6

UP = UNPROTECTED  
 P = PROTECTED  
 \* = Baseline bilirubin value was 130umol/l.

The finding that unconjugated bilirubin is more labile than conjugated bilirubin at room temperature (25°C) and in exposed specimens at 4°C agrees with reports elsewhere (Tietz *et al* 1994) but contradicts Cremer *et al.* (1964). However, at low temperature in the dark conjugated bilirubin was more labile, a finding likely to arise from greater stability of unconjugated bilirubin in conditions unfavourable to solubility (Tietz *et al* 1994) (4°C in this case).

The rate of bilirubin lability throughout the 12 hours of storage was not constant. Generally, zero hour was 10.00am and the experiment ended by 10pm (12 hours). The rate of lability was observed to be higher and near-constant during the first 3 hours (10.am - 1pm) when sunray was intense and room temperature higher. Fig. 1. The rate dropped during the evening and night when fluorescent bulb provided artificial light. The rates obtained in this study could be used for correction of bilirubin values. Bilirubin loss can be corrected by reliance on data (% loss ) presented in Table 1. Requirements for correction are knowledge of time of blood sampling, means of transportation of blood to laboratory (protected or unprotected at 25° C or 4°C) and time of laboratory analysis. This study has provided a chart (Table 1) to correct for effects of time, light and temperature emanating from delay between blood sampling and analysis for bilirubin in Calabar and any other geographical location with similar climatic conditions. Such correction would enhance reliance on bilirubin results in monitoring icteric patients.

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