

Stability of Human Sera Collected for Clinical Chemistry Determinations

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Two vital steps in any chemical analysis of biological fluids are the collection of the specimen from the patient and its preservation from the time of collection until the actual examination is begun. There are many potential sources of error, beginning with the securing of the specimen and not ending until the results are placed in the patient's record. It is not within the scope of this report to consider all facets of potential error. The work of Caraway (1) and Wirth and Thompson (2) consider these in some detail, including the effect of medications on clinical chemical laboratory results.

Since laboratory centralization is coming more and more into being in this country, the shipping of specimens to central laboratories has become more frequent and extensive each year. In spite of this trend, it is very difficult to obtain documented information on the stability of specimens under the conditions of transport by mail and other means. There is a considerable variance in the literature on the stability of different substances in blood serum. For example, Mosley and Goodwin (3) found SGPT was very unstable at -20°C . but was relatively stable at low temperature (-50°C .) up to 98 days. Juul (4), on the other hand states that SGPT is stable at -20°C . for 8 days. In our laboratory it was found that SGPT could not be preserved at all in a -20°C . freezer. Tests were not made in our laboratory at -50°C . There are numerous other similar conflicts. Much of the stability testing has been done for periods of 10 days or less, which is satisfactory in most situations if specimens to be mailed or transported are handled with reasonable promptness.

Henry (5) annotates most of his procedures with a note as to the relative stability of the substance to be tested under the usual laboratory conditions of room temperature (25°C .), refrigerator (4°C .) and freezing (-20°C .). Hagebusch (6) discusses some of the practical problems arising in a central laboratory in the St. Louis area.

In the case of enzymes much of the testing has been done on sera containing normal levels of the specific enzyme with little attention to carefully controlled studies on abnormal levels (7). Information on the stability of some enzymes at room temperature is lacking.

The following chart represents an attempt at listing the stability of various substances under the conditions that exist in the routine clinical chemistry laboratory. Some of the times are at variance with those given in the various publications on the subject. They do represent an attempt to arrive at practical limits and incorporate data obtained in our

laboratory from a continuing study of the problem of specimen stability. Reliable published data is lacking on the effect of freezing at -20°C . in many instances. It is likely that some of the room temperature times and refrigerator stability times may be longer or shorter than shown based on individual or unpublished experience.

A perusal of these data indicate that for practical purposes refrigeration of serum specimens after collection will be satisfactory, unless the specimen is frozen. The time for handling a specimen to be analyzed for bilirubin is very short; a maximum of 4 days with care to avoid exposure to light being essential for reliable results, if not frozen. Glutamic pyruvate transaminase (SGPT) is very unstable and cannot be shipped unless the shipping time is carefully controlled and is less than two days under refrigeration. Glucose will decrease if sample left at room temperature. As noted below glycolysis can be reduced with a sodium fluoride thymol mixture.

Shipping is best accomplished by using insulated containers with cooling provided by commercially available cans or plastic bags than can be frozen. In a properly insulated container they will maintain a low enough temperature to preserve the specimen en route. Obviously, sera can be shipped without refrigeration for those tests that show a good stability at room temperature, i.e., a room temperature (25°C .) stability for 7 days or more. However, in shipping, particularly during the summer months, temperatures may exceed 50°C . No good information is available on the effect of such temperatures. Hagebusch (6) considers this and found urea nitrogen, creatinine, uric acid, protein and SGOT little affected by excessive heat in shipping. Glucose, albumin, globulin, bilirubin, alkaline phosphatase and cholesterol showed varying degrees of instability.

The use of "preservatives" for maintaining the stability of specimens en route to a central laboratory is used in some instances. The literature and standard textbooks that were consulted were rather mute on this point. 11 mg. of a 10:1 mixture of finely powdered sodium fluoride and thymol per ml. of blood will help prevent glycolysis. Doe, Mellinger and Seal (8) used a citrate buffer at pH 6.2 in serum to be tested for alkaline phosphatase and found this would lessen the decline that normally occurs at room temperature.

It appears that the whole area of proper specimen preservation needs more careful investigation with both normal and especially serum containing abnormal elements. The relative stability of most specimens handled in the ordinary hospital clinical laboratory present no particular problems. However, with more and more utilization of central facilities more specific information on specimen stability is needed. It is true a specimen can be shipped and the determination made for whatever substances that are of concern to the physician. However, are the results

always completely reliable? In the majority of instances they appear to be acceptable. All of the answers to this problem are not complete at this time and must await further and more systematic study.