A PROTOCOL FOR ADOPTING CLINICAL CHEMISTRY

METHODS: ADOPTION OF ASSAY TO MODEL

by

Leonard Gary Nielsen

A thesis submitted to the faculty of the University of Utah in partial fulfillment of the requirements for the degree of

Master of Science in Medical Technology

College of Pharmacy University of Utah

June 1977
THE UNIVERSITY OF UTAH GRADUATE SCHOOL

SUPERVISORY COMMITTEE APPROVAL

of a thesis submitted by

Leonard Gary Nielsen

I have read this thesis and have found it to be of satisfactory quality for a master's degree.

May 18, 1977
K. Owen Ash

I have read this thesis and have found it to be of satisfactory quality for a master's degree.

Date 18 1977
Sarah A. Wise
Member, Supervisory Committee
FINAL READING APPROVAL

To the Graduate Council of The University of Utah:

I have read the thesis of ___________________________ in its final form and have found that (1) its format, citations, and bibliographic style are consistent and acceptable; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the Supervisory Committee and is ready for submission to the Graduate School.

May 18, 1977

K. Owen Ash
Member, Supervisory Committee

Approved for the Major Department

Approved for the Graduate Council

M. McMurrin
Registrar, The Graduate School
ABSTRACT

A protocol is presented for adopting clinical chemistry methods in the service laboratory. The protocol may be used to adopt alternate procedures to those currently in use and for the adoption of new methods not previously performed by the service laboratory. The critical points in method adoption which require consideration are presented along with instruction for the resolution of each critical point. Following the protocol, a manual kinetic γ-Glutamyl Transferase assay method was adopted in the Clinical Chemistry Laboratory, University of Utah Medical Center. The steps taken, from the selection of assay conditions through the introduction of the new test to staff physicians, are described.
ACKNOWLEDGMENTS

I wish to express my gratitude:

To Dr. K. Owen Ash for his support and guidance as chairman of my committee.

To the other members of my committee: Dr. David Roll and Sarah A. Wise, M.S., for their support and guidance.

To the Department of Medical Technology for their friendship and assistance.

To the personnel of the Clinical Laboratories, University of Utah Medical Center.

This work was made possible by an Allied Health Traineeship Grant, Agency Number 1A iAO 2 AH 00479-01.
TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>iv</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>v</td>
</tr>
<tr>
<td>I. INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>II. A PROTOCOL FOR THE ADOPTION OF ANALYTICAL METHODS IN THE CLINICAL CHEMISTRY LABORATORY</td>
<td>3</td>
</tr>
<tr>
<td>Introduction</td>
<td>3</td>
</tr>
<tr>
<td>The Protocol: Critical Points of Method Adoption</td>
<td>4</td>
</tr>
<tr>
<td>Need for the Test</td>
<td>6</td>
</tr>
<tr>
<td>Literature Search and Interpretation</td>
<td>6</td>
</tr>
<tr>
<td>Method Selection</td>
<td>7</td>
</tr>
<tr>
<td>Selection of Assay Conditions</td>
<td>8</td>
</tr>
<tr>
<td>Standards, Reagents, and Blanks</td>
<td>8</td>
</tr>
<tr>
<td>Use of Controls</td>
<td>9</td>
</tr>
<tr>
<td>Linear Range</td>
<td>11</td>
</tr>
<tr>
<td>Sensitivity, Specificity, and Stability</td>
<td>12</td>
</tr>
<tr>
<td>Accuracy</td>
<td>13</td>
</tr>
<tr>
<td>Precision</td>
<td>15</td>
</tr>
<tr>
<td>Recovery</td>
<td>16</td>
</tr>
<tr>
<td>Interfering Factors</td>
<td>17</td>
</tr>
<tr>
<td>Verification of Literature Claims</td>
<td>18</td>
</tr>
<tr>
<td>Establishment of Reference Intervals (Normal Range)</td>
<td>18</td>
</tr>
<tr>
<td>Correlation with Reference Method</td>
<td>26</td>
</tr>
<tr>
<td>Correlation with Disease Conditions</td>
<td>20</td>
</tr>
<tr>
<td>Writing Procedure Manual – Technologist Training</td>
<td>20</td>
</tr>
<tr>
<td>Pricing the Test</td>
<td>22</td>
</tr>
<tr>
<td>Communication with Physicians – Soliciting Test</td>
<td></td>
</tr>
<tr>
<td>Utilization</td>
<td>23</td>
</tr>
<tr>
<td>Follow-up of Test Utilization and Performance</td>
<td>24</td>
</tr>
<tr>
<td>Conclusion</td>
<td>24</td>
</tr>
<tr>
<td>Bibliography</td>
<td>25</td>
</tr>
<tr>
<td>III. ADOPTION OF A MANUAL KINETIC γ-GLUTAMYL TRANSFERASE METHOD IN THE SERVICE LABORATORY</td>
<td>30</td>
</tr>
<tr>
<td>Introduction</td>
<td>30</td>
</tr>
<tr>
<td>Materials and Method</td>
<td>34</td>
</tr>
</tbody>
</table>
INTRODUCTION

Keeping abreast of technical advances, which are important to optimal patient care, is a continuing challenge to all clinical chemistry service laboratories. Constantly, we are presented with more advanced analytical methods which offer greater simplicity, specificity, sensitivity, precision, and accuracy over the existing methodologies. The service laboratory must be continually alert to these new developments and be prepared to evaluate and implement, for the service function, those assays which meet the needs of the physicians and their patients.

How can new methods be effectively and efficiently evaluated and established in the service laboratory? A systematic protocol for the selection, evaluation, and implementation of analytical methods is critical to the continuing quest for improved health care, and would ensure that analytical methods are properly introduced and controlled in the service laboratory. A complete protocol for the adoption of analytical methods in the service laboratory has not previously appeared in the literature.

A systematic protocol for the adoption of analytical methods in the service laboratory is herein presented. The protocol may be used not only for the adoption of alternate methods which may offer important advantages over existing methodologies, but also for the adoption of entirely new analytical methods.
The protocol appears in Section II as a separate paper in the format of the American Journal of Medical Technology, and is co-authored by Dr. K. Owen Ash, Director of Clinical Chemistry Laboratory, University of Utah Medical Center. Twenty critical points of method adoption are specified. A bibliographical discussion of each point is presented, along with the recommended procedure for treatment.

Following the protocol for adopting analytical methods in the service laboratory, a manual kinetic g-Glutamyl Transferase assay method was adopted for routine use in the Clinical Chemistry Laboratory, University of Utah Medical Center. The steps taken, from the selection of assay conditions through the introduction of the new test to staff physicians, appears in Section III as a separate paper in the format of the American Journal of Medical Technology. The paper is co-authored by Dr. K. Owen Ash.
A PROTOCOL FOR THE ADOPTION OF ANALYTICAL METHODS
IN THE CLINICAL CHEMISTRY LABORATORY

by

Leonard G. Nielsen and K. Owen Ash

Introduction

Keeping up to date with technical advances, which are important to optimum patient care, is a continuing challenge to all service laboratories. Constantly we are presented with more advanced analytical methods which offer greater simplicity, specificity, sensitivity, precision and accuracy over the existing methodologies. How can new methods be effectively and efficiently established in the service laboratory? The protocol which we present was designed to provide a systematic approach to method implementation in the service laboratory. A complete protocol has not previously appeared in the literature. Many reports, however, have stressed the importance of certain aspects of the proposed protocol.

In 1975 the National Committee for Clinical Laboratory Standards (NCCLS) presented an excellent protocol for establishing precision and accuracy of automated instrument systems. Their treatment was specifically aimed at evaluating automated instruments in terms of precision and accuracy.
In 1975 Rodgerson and Tietz\textsuperscript{33} stressed the importance of adoption of standardized methods in all clinical chemistry laboratories. They proposed that all methods adopted by laboratories be based on sound experimental data obtained by following an agreed-upon protocol. However, they did not present a protocol. Several critical points in the evaluation of a new method were suggested by Acosta,\textsuperscript{1} in 1975, but again a protocol was not presented.

An effective protocol for the adoption of laboratory methods is critical to our continuing quest for improved health care. A protocol for the evaluation, adoption, and implementation of new analytical methodologies in the clinical chemistry laboratory is presented. Our protocol may be used not only for the adoption of entirely new test procedures, but also for the adoption of alternate methods which may offer important advantages. The protocol includes twenty different points which should be considered. This report includes a bibliographical discussion of each point along with our recommended procedure for treatment.

Because spectrophotometry is used in the bulk of clinical chemistry procedures, the following discussion emphasizes spectrophotometric procedures. However, the concepts presented can directly be adapted for use with other analytical techniques.

The Protocol: Critical Points

of Method Adoption

Before any analytical method may be considered for patient care in a service laboratory the following points must be considered:
Need for the Test

Literature Search and Interpretation

Method Selection

Selection of Assay Conditions

Standards, Reagents, and Blanks

Use of Controls

Linear Range

Sensitivity, Specificity, and Stability

Accuracy

Precision

Recovery

Interfering Factors

Verification of Literature Claims

Establishment of Reference Intervals (Normal Range)

Correlation with Reference Method

Correlation with Disease Conditions

Writing Procedure Manual - Technologist Training

Pricing the Test

Communication with Physicians - Soliciting Test Utilization

Follow-up of Test Utilization and Performance

We present these points as the protocol for methodology adoption in the service laboratory. The format for satisfying the requirements of each point, along with bibliographical discussion, is now presented.
Need for the Test

Regardless of whether or not a particular test has ever been previously utilized in the service laboratory it is impractical to consider the adoption of an assay method unless it will yield relevant and useful information to the physician. It must be determined if the physicians served by the laboratory would utilize the assay should it be adopted. Is there sufficient need for the test? Will the usefulness of the test’s results justify the cost of the test to the patient?

Too frequently laboratory personnel go through the effort of providing a new test only to later discover there is little need for the test among the physicians they serve. What constitutes a sufficient need must be determined by the individual laboratory for the particular assay in question. Unless the nature of the assay dictates that it must be performed on site, we suggest that infrequently used tests might best be sent to a reference laboratory until the need for the test is sufficient to justify adoption of the test according to the recommended protocol.

Literature Search and Interpretation

Once the need for the test has been documented, a literature search should be conducted to determine what assay methods have been previously presented. If a commercial method is being considered, the references cited should be reviewed as to the development, characteristics, and limitations of the method. In the interpretation of scientific articles, the points reported by Barnett⁴ and Henry, et al.,¹⁹ should be considered:
1. Are the materials and methods clearly described?
2. Are the reagent and sample stabilities specified?
3. Are the effects of hemolysis, pH, and temperature discussed?
4. Are the sample and collection requirements stated?
5. Were an adequate number of samples included in each experiment?
6. Was a conscious effort made to avoid sampling bias?
7. In precision studies were there outlier values, i.e., were values found greater than +3 SD of the mean? If so, how were they treated?
8. Was statistical analysis necessary? Was it used? Were the proper statistical tests used and were the calculations correct?
9. Are the conclusions valid in view of the data?

Method Selection

A method under consideration should be rejected if the precision data or the results of analysis of normal individuals yield greater than three outlier values in 40 analyses, or if the coefficient of variation (CV) is greater than 8%, as this demonstrates the existence of too many dangerous random errors.\(^4,35\)

We agree with other investigators that existing "selected" or "reference" methods should have priority in consideration for adoption over other available methods.\(^15\) However, the question has been asked as to what society or agency has the authority to declare international selected or reference methods?\(^9,29\) Clearly, this problem has not been resolved and requires world-wide participation and cooperation. Ideally, the selection of any assay method should be
based upon reliability, accuracy, precision, and clinical usefulness -- not upon the ease of adoption. Primary considerations in method selection must include: (1) The sample size required; (2) speed of analysis; (3) personnel and expertise required; (4) equipment and reagents required; (5) accuracy, precision, and overall reliability; and (6) assay conditions.

**Selection of Assay Conditions**

For the adoption of selected or reference methods this task has already been completed. However, in modifications of these or other methods, several problems must be resolved. For assays intended for pediatric use the volume and type of specimen is a prime consideration. The temperature under which the assay is performed is another consideration; assays requiring temperatures of 25°C may not be satisfactory due to the difficulty in accurately maintaining this temperature. The room temperature in many laboratories may exceed this. Laboratories should comply with CAP (College of American Pathologists) recommendations for laboratory conditions. In all cases the assay results must be in standard units of measurement that allow some degree of correlation with assays performed in other service laboratories.12

Assay conditions should, whenever possible, be selected so that the assay reaction is linear with respect to the concentration of the substance being analyzed.

**Standards, Reagents, and Blanks**

Before attention is focused on an assay method, it is imperative that
certified physical standards be used to check-out the analytical equipment
used in the laboratory, especially absorbance sensitivity and wavelength
calibration. Whenever possible, certified standard material and
analytical grade reagents should be used in the preparation of assay
standards and reagents. For many enzyme assays suitable standard
materials do not exist. This problem will be discussed in a later
section.

We recommend that a suitable number of standards (depending on
the linearity of the assay) be included in each assay run. If used, cali-
bration curves must be closely scrutinized and continually evaluated.

The reagents used in the assay must be chemically pure and
stabilities must be specifically determined. If the reagents contain
interfering chromagens or are subject to non-specific reactions during
the assay, a reagent blank must be included in each assay run to correct
for such errors. In end-point assays reagent and serum blanks may need
to be included in each assay to correct for non-specific absorbance
changes caused by spontaneous substrate breakdown and/or icteric,
lipemic or hemolyzed samples. For each assay the appropriate number of
standards, reagent blanks, and sample blanks which will provide quality
assurance must be determined.

Use of Controls

We cannot stress strongly enough the importance of including
control samples in each and every assay run. These controls provide an
ongoing quality control check on the overall assay, including equipment, reagents, and assay conditions. Many investigators\(^\text{15,28,38}\) suggest that three controls (low normal, high normal, and abnormally elevated) must be used. We feel that as long as the assay method has been proven to be linear over the entire range of expected values, as found in normal and disease conditions, a minimum of two controls (normal and significantly abnormal) is sufficient for within-run quality control. These controls must be randomly spaced among the patients' specimens. Additionally, we recommend the inclusion of "blind control samples" (coded as patients) randomly included with the patients' specimens to reduce technologist bias towards the means of the target ranges.\(^7\)

When suitable commercially prepared control material is not available or if the concentrations of the constituents in question (hereafter referred to as "analytes") are not adequate regarding normal and abnormal values, the laboratory may prepare its own control material. Bowers, et al.,\(^7\) have presented a comprehensive format for the preparation of excess patient sera for use as assay control material. We have found that commercially available control sera are usually suitable for use in the normal range. If commercial control serum is not adequate in the abnormal range, this can be made by adding weighed amounts of the analytes to commercial control serum, by diluting the control serum with a protein-containing diluent, or by collecting a large volume of excess patient sera from patients in disease states that would yield
clearly abnormal levels of the analytes in question. This serum pool should be frozen, thawed, and refrozen several times (to eliminate labile components), centrifuged, filtered, and dispensed into many small aliquots as needed for each day's workload. Before use, this control material must be tested for Hepatitis Associated Antigen with negative results. Nevertheless, laboratory personnel should treat the control sera with the same precautions afforded to patient sera.

Before being put into routine use, the range of acceptable values of the control serum pool must be determined. The method of doing this, and the sample size necessary, is not consistently presented in the literature. We suggest performing no more than two analyses per day until a minimum of 60 values have been obtained. This should take into account variations in technologist technique, temperature, reagents, and instrumental precision. The acceptable range of values would then be taken as \pm 2 SD of the calculated mean, after all values fall within \pm 3 SD. The treatment of "outlier" values is presented in the precision discussion. Regular evaluation and re-assignment of the confidence intervals is necessary.

**Linear Range**

The need for the determination of the linearity of assay methods is clearly documented. Knowledge of the linear range tells the technologist when abnormally elevated specimens must be diluted and reanalyzed. For end-point colorimetric assays, a minimum of six
certified standard solutions representing concentrations ranging from the below normal through at least a ten-fold elevation above the normal range should be assayed in triplicate. The means of the resulting absorbance changes should be examined as a function of concentration to determine the linearity of the assay. The linearity determinations must be made on the assay exactly as it is performed for obtaining patients' values.

For kinetic two-point enzyme assays, the change in absorbance due to disappearance of substrate or appearance of enzymatic products as a function of time should be used to determine linearity. If the reactions are not linear, sample volumes and/or substrate concentrations can often be adjusted to obtain a suitable linear range. For assays that are not linear, such as certain radioimmunoassays, it is mandatory to run enough standards and/or controls to reconstruct the curve for each assay run.

When the assay is in actual use, values greater or less than the experimentally determined linear range must receive the necessary attention by the technologist; how these values are handled should be specified for each assay.

Sensitivity, Specificity, and Stability

Whenever possible, assay methods must have sufficient sensitivity to accurately detect concentrations of analytes both below and at least ten times greater than the normal range of values without necessitating sample dilution, and without resulting in significant carry-over to other samples within the run. This degree of sensitivity can often be obtained by modification of the sample/substrate reagent ratio or adjustment of the substrate
concentration.

In addition, the assay method must be specific for the analyte under analysis. The results must not be significantly influenced by other common sample or reagent constituents. The assay should be monitored at a wavelength which yields maximum absorbance of the assay product with a minimum absorbance of non-specific chromagens.

It is important to stress that specificity is not an absolute entity. Glucose oxidase is regarded as being specific for glucose. However, uric acid is known to compete with the chromagen of that assay for peroxidase activity.\textsuperscript{14} Also, many certified enzyme materials are of non-human origin and have been reported to behave differently than the same enzyme of human origin.\textsuperscript{4}

Stability of both the reagent and specimen must be determined at room temperature, refrigerator temperature, and upon freezing, as a function of time. Varying protocols for the determination of stability have been presented.\textsuperscript{32,37} We suggest the use of the criteria proposed by Thiers, \textit{et al},\textsuperscript{37} for the determination of both substrate reagent and sample stability. Stability is confirmed if the change in the mean of a subsequent analysis is less than $\pm 1$ SD of the original mean.

\textbf{Accuracy}

Accuracy and precision should be considered independently; very precise methods may have poor accuracy.\textsuperscript{27} The accuracy of any assay method must be thoroughly evaluated and documented.\textsuperscript{1} The use of
analytical grade reagents and certified standard materials are prerequisites of assay accuracy. 12, 15

Accuracy may be determined by comparison with a reference method, recovery experiments, and/or by analysis of assayed reference materials. Both commercial assayed samples and proficiency test samples have been used to assess accuracy. When using assayed materials to assess accuracy we recommend at least twenty determinations of each of three reference materials representing low normal, high normal, and elevated concentrations of the analyte. Assay accuracy can be expressed as a percentage, as follows:

\[
100 = \% \text{Accuracy} = \left(1 - \frac{\text{mean value} - \text{actual value}}{\text{actual value}}\right) \times 100
\]

Therefore, when accuracy is 100% the assay value is in agreement with the accepted reference value. Accuracy must be determined over the usable concentration range of the assay.

After assay methods have been properly established in the laboratory, accuracy can be monitored by comparing control sera values with the results of interlaboratory proficiency surveys. 27

Enzyme assays present additional problems as certified standard materials are rarely available and accepted reference methods may not exist. For such enzyme assays, precision and reference interval studies must replace accuracy determination. 4, 34
Precision

The precision of the assay method must be established at no less than two concentrations: Normal and elevated levels. Both the within-run and day-to-day precision of the assay must be documented. The literature offers no agreement as to the minimum sample size for precision analysis. We recommend that the within-run precision study include not less than 30 replicate analyses on each of at least two concentrations, and that these samples be assayed in random order. The day-to-day precision study requires at least 40 replicate analyses for each concentration level, with no more than one set of data used per assay run, and no more than two runs per day recorded. This will take into account random variables such as temperature, reagents, instrument precision, and technologist technique, etc.

The SD and coefficient of variation (CV) are meaningful measurements of precision only when one is dealing with random analytical errors that are normally distributed. When calculating the SD, values that fall outside of $\pm 3$ SD of the mean are termed outlier values and should be excluded from calculations as they represent error other than random analytical error; errors due to contamination, pipetting, transcription calculations, etc. However, because these outlier values represent errors that would also alter patient results they cannot be forgotten. Outliers should be specified along with the SD, as a percent of the total values involved.
We support the recommendation of Sparapani, et al., that analytical methods with a CV greater than 8%, or with outlier values exceeding 8%, should not be considered for adoption, as they may possess too many dangerous sampling errors. However, at the present time many laboratories may not have the equipment necessary to control certain enzyme assays to a CV of 8% over the range of the assay. Several of these problem assays are necessary for patient care and should therefore not be discontinued. Every effort should be made to improve the precision of these assays rather than merely accepting their inadequate precision.

Recovery

Recovery experiments support the accuracy of an assay, but 100% recovery does not necessarily indicate 100% accuracy. For example, chlorine contamination in uric acid assays forms hypochlorite, which oxidizes a constant amount of uric acid; recovery may be 100%, but the assayed value will not be accurate.

To document the recovery characteristics of a non-enzyme method, a suitable standard material may be weighed out and added, in varying amounts, to control sera. We suggest 20%, 50%, and 100% concentrations above the base concentration of the control sera. These "spiked" samples are to be assayed in triplicate, and the mean values calculated. The recovery is expressed as a percentage of the true (expected) concentration.
\[
\left[ 1 - \frac{\text{mean value} - \text{true value}}{\text{true value}} \right] \times 100 = \% \text{ Recovery}
\]

To determine the recovery characteristics of enzyme assay methods, appropriate mixtures of normal and elevated patient sera of known concentration or activity may be analyzed and treated in a similar manner.

**Interfering Factors**

A complete discussion of the many factors which can interfere with a specific assay is beyond the scope of this paper. However, interferences resulting from certain commonly experienced factors must be examined.

For example, the influence of hemolysis, bilirubin, common laboratory anticoagulants, and capillary vs. venous blood samples must be documented at both normal and elevated concentrations. This may be done by dispensing several appropriate patients' sera into aliquots. On each patient's sample, baseline values are determined prior to addition of possible interfering substances. Aliquots of the assayed sera are then spiked with known amounts of the potential interferent and reassayed to determine the extent of the interference.

For hemolysis studies the respective patient's red blood cells are added to his or her serum; this is then frozen and thawed to produce hemolysis. Anticoagulant effects can be assessed by testing plasma samples treated with EDTA, heparin, oxalate, and citrate.
Triplicate analysis of each sample is sufficient to detect gross interference. If statistical analysis is necessary, at least 30 samples should be included. A t-test may be used to determine statistical significance between the baseline values and the values obtained in interference studies. 19, 21

Verification of Literature Claims

All too often service laboratories begin routinely using new methods for patient care without any form of evaluation or documentation as to accuracy, precision, or reference intervals. This is especially true of commercially prepared "kit" methodologies. Rodgerson and Tietz 33 have suggested that each laboratory should independently perform all experiments in the adoption of a new assay method. While we agree with the principle of this suggestion, independently performing all the experimental work in the development of a method that has been previously thoroughly documented may be unnecessary. Any properties or characteristics pertaining to the method which have not been adequately documented, however, will have to be investigated by the service laboratory. 13, 15

In any case, the service laboratory will have to perform its own experiments to document precision, accuracy, sample stability, and reference intervals.

Establishment of Reference Intervals

(Normal Range)

The concept of normal values has been challenged by many. Since
there is no definition of what a "normal" individual is, it has been recommended that the term "normal value" be replaced by "reference interval." Various methods have been presented regarding the establishment of reference intervals in the service laboratory.

The use of blood bank donors for establishing reference intervals has particular merit; the donors are presumably healthy, and serum specimens are easily obtained. Reference intervals should differentiate sex and age, if possible, and a minimum of 40 individuals should be included in each group. If a near-gaussian distribution is not found, or if the distribution of values is markedly skewed, more samples must be included in the study. The reference intervals may be taken as ±2 SD of the mean, after exclusion of any outlier values.

The reference intervals must be periodically rechecked to ensure current validity. The reference range is probably skewed with periodic fluxuation of circadian and diurnal rhythms. The Chi Square test may be of value in this determination.

Harris submits that reference intervals may be useful only for the first screening of a hospital patient. The patient's own previous values later serve as a reference. It is therefore important to define exactly what constitutes a significant change in a patient's values in view of the random analytical error of the assay method. We submit that a change equivalent to ±4 SD, based on the day-to-day precision of the assay, unquestionably denotes a significant shift, and is due to conditions other than random
analytical error. Changes of less than $\pm 0.4$SD may have clinical significance, but may be due to random analytical error.

Correlation with Reference Method

The proposed assay method should be compared to established reference methods whenever possible. In order to successfully make this comparison all units of measurement and reaction conditions (pH, temperature, direction of reaction, etc.) must be the same.\textsuperscript{1,4,34} A coefficient of correlation may be obtained from the analysis of at least 40 patients' samples by both methods.\textsuperscript{21} If poor correlation is found, it must then be determined which method is actually in error.

Correlation with Disease Conditions

The correlation of assay values with various disease conditions must be documented.\textsuperscript{1,19} For many assays this data may be found in the literature. If not, the service laboratory must experimentally determine this correlation by analysis of no less than 100 patients in appropriate disease conditions, including patients that should demonstrate relatively "normal" assay values. Even though clinical studies may have been reported in the literature, the service laboratory should conduct experimental work to demonstrate the expected ranges of values in appropriate disease states for the assay method in its own laboratory.

Writing Procedure Manual - Technologist Training

The adoption of an assay method in the service laboratory requires
that a complete and concise procedure be written, verified, and included in the laboratory's procedure manual.  Although the format of procedure manuals may differ among laboratories, it must include all pertinent references, step-by-step instructions on how to perform the assay, sample specifications including collection and handling techniques, equipment to be used, wavelength and slit width settings, and known interfering factors. Also included must be specifications for the type and number of standards, blanks, controls, calculations required, and a list of the common sources of error, along with a troubleshooting discussion for solving common problems that may be encountered.

McSwiney and Woodrow propose that even in the best laboratories 3% of everything done is in error. No assay, regardless of how accurate and precise it may be, will yield useful results unless the technologist performing the assay is thoroughly trained. The technologists performing the assay must understand the clinical significance of assay values as well as all critical points of the methodology which may result in analytical error. We have found that an effective way to teach the necessary assay techniques is to personally present a synopsis of pertinent information regarding the assay, and then "walk-through" the assay several times pointing out critical points needing special consideration. After this, the technologists are allowed to experiment with the new assay on control sera and preselected patient samples until they have acquired the expertise and confidence necessary to completely perform the assay.
Pricing the Test

It is not within the scope of this paper to present a complete dissertation of the theory and techniques of cost accounting. An abbreviated, but comprehensive treatment of financial management of the clinical laboratory has been presented by Bennington, et al.\textsuperscript{6}

The policies of cost accounting, for many service laboratories, are predetermined by their administrations. However, there are some principles of test costing that apply in nearly all situations and a discussion of these principles is warranted.

We have found that the common methods of test pricing are: (1) Market pricing (what the market will bear), (2) competitive pricing (what other laboratories are charging), and (3) cost-plus pricing (actual cost of the test plus charges for overhead, pathologist’s services, salaries, uncollectable past-due accounts, etc.). The latter seems to be the most likely to ensure the financial success of the service laboratory without unnecessarily raising patient costs.

The methods of test cost analysis and the policies of allocating indirect costs (overhead, depreciation of equipment, administrative costs, etc.) vary markedly among laboratories. In view of this, a discussion of "differential" cost analysis may be helpful regardless of accounting policies. The differential cost analysis is specifically finding the actual (direct variable) cost of adding an additional test in the laboratory, independent of all other laboratory expenses.
Direct variable costs include the costs of standards, controls, reagents, expendable supplies, (e.g., test tubes, report slips, blood collection supplies) and technologist time for the actual assay performance and reagent preparation. In addition, losses due to spoilage of prepared reagents must also be considered. From this data the differential cost per test may be accurately determined. This differential cost (direct cost) per test, along with the fixed laboratory expenses and allocations for a given period of time will enable the break-even point (minimum number of tests that must be performed in a given time period to realize a profit) to be determined by plotting revenue against total expenses on a break-even graph.\(^6\)

**Communicating with Physicians**

- **Soliciting Test Utilization**

The laboratory test ordering practices of physicians vary little from when they were interns.\(^6\) Physicians generally care little about the technical capabilities of an assay method, but they are very much concerned about how useful the test is to them as a diagnostic tool. Kronfield\(^6\) has suggested that the pathologist making rounds, and laboratory newsletters explaining new tests, are two useful tools in communicating with the physicians. If the pathologist is aware of the capabilities of a new test and feels it is of significant diagnostic use, he can suggest test utilization to other physicians for appropriate patients encountered on medical rounds. It is the responsibility of the laboratory to see that the physicians and
pathologists have this information. Laboratory newsletters are useful for introducing the availability of a new test, along with the benefits and limitations of the test as a diagnostic tool. The laboratory must clearly define what constitutes a significant change in a patient's assay values, and what the reference intervals are for that specific laboratory.

Follow-up of Test Utilization and Performance

Once an assay method has been adopted for patient care in the service laboratory, the new assay must continually be evaluated in terms of clinical usefulness. Close communication with the pathologists and physicians ordering the tests is extremely important, not only to assess the value of a particular test, but also to monitor overall effectiveness of the laboratory.

It has been our experience that too frequently assay method inadequacies are not discovered by laboratory personnel until complaints of error are made by the physicians. Close communication with the physicians enables the laboratory to detect methodology error in the early stages and will increase physician confidence in the service laboratory.

Conclusion

The protocol presented is of significant value to service laboratories in effectively adopting new analytical test methods, in that it offers a standardized, systematic approach to methodology adoption, starting with the assessment of need for a test and continuing through evaluation of the
assay's utility to the physicians.

Additional investigation in many areas, such as technologist training techniques, effective communication techniques between the physicians and the laboratory, and the establishment of international definitive and reference methodologies is necessary and urged. However, until improved techniques are available, our protocol is a viable tool to the service laboratory.
Bibliography


ADDITION OF A MANUAL KINETIC G-GLUTAMYLYL TRANSFERASE METHOD IN THE SERVICE LABORATORY

by

Leonard G. Nielsen and K. Owen Ash

Introduction

As a general rule, assays offered by clinical service laboratories do not represent unique contributions to scientific knowledge. Usually the new methods have been discussed in scientific meetings and published reports before the methods receive sufficient recognition to have utility in service laboratories. Even though such methods are not unique contributions to our state of knowledge, they nevertheless are "new" methods for the service laboratory; a new method being one that is not presently available in the laboratory. Because of the continued rapid progress and widespread interest in the field of laboratory testing, many new developments will be generated by those outside the laboratory; yet, it is extremely important that a research and development effort be maintained as a part of the service laboratory to maintain viability.

The service laboratory must be continually alert to new developments and be prepared to implement, for the service function, those assays
which meet the needs of the physicians and their patients. A systematic format for selection and implementation of methods will insure that methods are properly introduced and controlled in the service laboratory. The method herein described was adopted in our service laboratory and is a model of our protocol for methodology adoption, which is presented elsewhere. 17

\[ g\text{-Glutamyl Transferase (g-GT)(EC 2.3.2.2) activity elevates in hepatobiliary and pancreatic disorders, and thus provides an assay for confirmation of these disorders.} \]

Although many tissues contain g-GT, serum g-GT originates primarily from the liver, pancreas, and kidney. 19 Kidney diseases rarely elevate serum g-GT, while hepatobiliary and pancreatic disorders may yield large elevations in serum g-GT activity. 19

Serum elevations of g-GT are usually indicative of hepatobiliary or pancreatic disorders; however, congestive heart failure, diabetes, respiratory disease, and myocardial infarction have also been reported to elevate g-GT activity. 19 In addition, elevations in g-GT may be found in epilepsy, radiotherapy treatment, and in patients being treated with enzyme-inducing drugs, such as phenobarbitone, warfarin, nitrazepam, methaqualone, 19 and antibiotics such as tobramycin and kanamycin. 3,20

Serum g-GT, however, is not elevated in bone disorders where metastasis to bone is absent, 9 nor is it elevated in normal adolescence and pregnancy, 15 where other enzymes, especially alkaline phosphatase, may
be elevated. Therefore, g-GT may be used to differentiate hepatobiliary and pancreatic disorders from bone disease in cases having elevated aspartate transaminase (AST), lactic dehydrogenase (LDH), and alkaline phosphatase activities.\textsuperscript{9, 19} g-GT is especially useful as a diagnostic aid in the initial stages of hepatobiliary disease and in alcohol-induced liver disease, since g-GT will elevate prior to bilirubin, AST, and alanine transaminase (ALT).\textsuperscript{19, 20}

In the assay selected for implementation in our service laboratory, g-GT catalyzes the transfer of the g-glutamyl group of the substrate, g-glutamyl-\(p\)-nitroanilide, to the acceptor molecule, glycyglycine. The reaction liberates \(p\)-nitroaniline, a yellow-colored compound, which is monitored at 405 nm. Figure 1 demonstrates the net reaction; a more detailed reaction mechanism has been reported by Karkowsky, et al.\textsuperscript{14}

Several methods for g-GT assay have been reported. Szasz described a kinetic method using g-glutamyl-\(p\)-nitroanilide as the substrate and glycyglycine as the acceptor molecule, in 1969.\textsuperscript{23} His method utilized an amnediol-HCl buffer at a pH of 8.2, a reaction temperature of 25\(^\circ\)C; the reaction was initiated by the addition of serum to the equilibrated reaction mixture. Numerous modifications of this method have been described. Rosalki and Tarlow recommended addition of concentrated substrate to buffered serum to initiate the reaction.\textsuperscript{24} Bondar and Moss\textsuperscript{5} described an enhancing effect on g-GT activity due to elevated serum levels of glutamic acid. They recommended the addition of 1 mmol/liter
Figure 1. Net reaction catalyzed by g-GTP in reaction mixture
of glutamic acid to the substrate to mask the effect of elevated serum glutamate levels. Commercial kits are available that utilize the same substrate reagent, reaction conditions, and similar procedures as that used by Bondar and Moss.\(^5\) (Bio-Dynamics/bmc, Indianapolis, IN, catalog #15794, 16312; Worthington Biochemical Corp., Freehold, NJ, "Statzyme, g-GTP").

After reviewing the published methods we selected a manual kinetic method which with some modifications follows quite closely the method of Bondar and Moss.\(^5\) This simple method has been established in the service laboratory at the University of Utah Medical Center.

Eighty-five hospitalized patients with a variety of clinical conditions were monitored in order to confirm its utility in our patient population. The assay requires only 50 ul of serum and utilizes equipment which is normally available in most clinical laboratories.

**Materials and Method**

**Apparatus**

1. Bausch and Lomb Spectronic 100 spectrophotometer with flow-through cuvette (Bausch and Lomb, Inc., Rochester, NY).

2. Beckman Expandomatic SS-2 model 76 pH meter with a #39030 combination electrode (Beckman Instruments, Fullerton, CA 92634).

4. Falcon disposable culture tubes, 13x100 mm (catalog # T-1358-13A, Scientific Products).

Materials

1. g-L-Glutamyl-p-nitroanilide (G-6379, Sigma Chemical Co., P. O. Box 14508, St. Louis, MO 63178).

2. Glycylglycine, mw 132.1 (G-1002, Sigma Chemical Co.).

3. L-Glutamic Acid, mw 147.1 (G-1251, Sigma Chemical Co.).


Substrate-Better Reagent Preparation

Place 0.1068 g of g-L-glutamyl-p-nitroanilide in a 100 ml volumetric flask. Add 10.2 ml of 0.05 mol/liter HCl and swirl until substrate is entirely dissolved. Next, add 2.2400 g of Tris(hydroxy-methyl)aminomethane and dissolve with the addition of 50 ml of distilled water. Add 0.5284 g of glycylglycine and 0.0147 g of L-glutamic acid, then swirl until completely dissolved. Adjust final volume to 100.0 ml with distilled water. At 25°C the final pH should be 8.20 ± 0.05. If not,
adjust with either 6 mol/liter HCl or 6 mol/liter NaOH. Dispense into 3.0 ml aliquots and use within 24 hours (as long as the blank absorbance is less than 0.600) or immediately freeze at -20°C. The finished substrate-buffer reagent contains, per liter, 185 mmolcs Tris(hydroxymethyl)aminomethane, 4.0 mmoles of g-glutamyl-p-nitroanilide, 40 mmoles of glycylglycine, and 1.0 mmole of glutamic acid.

To prepare frozen substrate for use, thaw aliquoted reagent tubes at 37°C for 20 minutes, then place in a 56°C waterbath for ten minutes to redissolve all substrate components. The substrate-buffer reagent has been found to be stable for up to 24 hours at room temperature and for at least one month when frozen at -20°C.

**Procedure**

1. Pre-incubate sufficient tubes containing the substrate preparation for 15 minutes at 30°C; one tube for reagent blank, tubes for selected controls, and one tube for each patient serum.

2. For each tube (except the reagent blank) add 50 ul of serum and mix by inversion five times. Pre-incubate the reaction mixture two minutes at 30°C.

3. Zero the spectrophotometer (set at 405 nm) on distilled water, then read the absorbance of the reagent blank and of each sample. The flow-through cuvette should aspirate 0.8 ml of the test mixture for each reading.
Sample readings should be spaced 30 seconds apart to permit accurate timing.

4. After a reaction period of exactly five minutes, again determine the absorbance reading of each tube. The absorbance change of the reagent blank is used to correct for any non-enzymatic breakdown of substrate.

5. Subtract the change in absorbance of the reagent blank over the five-minute period from the respective five-minute absorbance change of each sample. Then multiply the change in absorbance over the five-minute period by 1232 to obtain the g-GT activity in International Units per liter (U/liter) at 30°C. The derivation of the conversion factor is detailed below:

Activity

One International Unit (U/liter) of enzyme activity is the enzyme activity that will liberate one umole of p-nitroaniline per liter of serum per minute. The molar absorptivity of p-nitroaniline at 405 nm is $9.9 \times 10^3$ liter-mole-cm. The molal absorptivity of p-nitroaniline at 30°C is as follows:

$$U/liter = \frac{\Delta \text{Absorbance (5 minutes)}}{(9.9 \times 10^3 \text{ liter/mole-cm}) (1 \text{ cm})} \times \frac{10^6 \text{ umoles}}{\text{mole}}$$

$$\times \frac{3.050 \text{ ml}}{0.050 \text{ ml}} \times \frac{1}{5 \text{ minutes}}$$

$$U/liter = \Delta \text{Absorbance (5 minutes)} \times 1232$$
Sample Calculation:

<table>
<thead>
<tr>
<th>Reagent blank: Final</th>
<th>.445</th>
<th>Patient: Final</th>
<th>.651</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>-.444</td>
<td>Initial</td>
<td>-.550</td>
</tr>
</tbody>
</table>
Absorbance change:  | .001 |                   | .101 |

\[
0.101 - 0.001 \text{ (blank)} = \text{net absorbance change of 0.100}
\]

\[
U/\text{liter} = 0.100 \times 1232
\]

\[
U/\text{liter} = 123
\]

**Results**

This method was found to be linear to at least 1100 U/liter, as shown in Figure 2. Based on ten different assays, the recovery of this method was found to be 100% at an activity level of 170 U/liter. Day-to-day precision studies on pooled human sera in both the near-normal and elevated ranges yielded the following precision data, at 95% confidence intervals:

<table>
<thead>
<tr>
<th></th>
<th>Pool 1</th>
<th>Pool 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean:</td>
<td>40 U/liter</td>
<td>136 U/liter</td>
</tr>
<tr>
<td>Standard Deviation: (+)</td>
<td>3.2 U/liter</td>
<td>5.8 U/liter</td>
</tr>
<tr>
<td>Coefficient of Variation:</td>
<td>8.0%</td>
<td>4.3%</td>
</tr>
<tr>
<td>Sample Size:</td>
<td>69 daily assays for each pool</td>
<td></td>
</tr>
</tbody>
</table>

Within-day precision studies on the same serum pools previously mentioned were as follows:

<table>
<thead>
<tr>
<th></th>
<th>Pool 1</th>
<th>Pool 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean:</td>
<td>40 U/liter</td>
<td>136 U/liter</td>
</tr>
</tbody>
</table>
Figure 2. Graphical representation of observed change in absorbance per minute of reaction time depicting a linear absorbance change of 0.891A, which in a 5 minute reaction period would represent an activity of 1100 U/liter. (Initial absorbance taken following a two minute incubation). Substrate exhaustion occurred in 60 minutes at an absorbance of 3.040.
Standard Deviation: (±) 1.4 U/liter 4.3 U/liter
Coefficient of Variation: 3.7% 3.0%
Sample Size: 30 samples 30 samples

The reference intervals determined by analysis of 96 healthy Red Cross blood donors were 0 to 30 U/liter for males, and 0 to 27 U/liter for females. Both groups simulated a gaussian distribution, but were slightly skewed to the left. The reference intervals were considered to be ±2 standard deviations (SD) of the respective means. We have presented elsewhere that a change in activity equivalent to ±4 SD, based on the day-to-day precision of the assay, unquestionably denotes a significant shift, and is due to conditions other than random analytical error. Changes of less than this may have clinical significance also, but may merely reflect random analytical error. Based upon this, we determined that an activity of 43 U/liter or greater denoted a clinically significant elevation in activity. Insufficient data was available for age differentiation in reference intervals.

We were unable to demonstrate any effect on g-GT activity due to hemolysis or elevated bilirubin levels up to 20 mg/dl. In addition to serum samples obtained by the recommended venipuncture technique, g-GT levels in sera from capillary blood and plasma containing EDTA, heparin, citrate, and oxalate/fluoride were compared. No significant effect on enzyme activity was demonstrated.
Following the stability criteria suggested by Thiers, et al,\textsuperscript{25} in which the means of at least two analytical test runs must be within one SD of each other, serum \( \text{g-GT} \) activity was found to be stable at room temperature for at least four days, up to one week at \( 4^\circ \text{C} \), and for at least three months frozen at \(-20^\circ \text{C} \). Thawing and refreezing up to five times had no measurable effect on enzyme activity.

A clinical correlation study was conducted in our hospital to confirm the utility of \( \text{g-GT} \) among our patients. Eighty-five hospital patients were analyzed for \( \text{g-GT} \) activity. Selection of patients was based on disease diagnosis and also on the results of other assays ordered; patients having both normal and abnormal results were included. Our findings confirm that for our patient population the clinical correlation data reported in the literature is applicable.\textsuperscript{4, 7, 9, 11, 15, 19, 23} Of twenty-five patients with diagnosed hepatobiliary and pancreatic diseases, the \( \text{g-GT} \) activities were found elevated in 100\% of these patients. Additionally, in 68\% of those patients greater elevations were observed in \( \text{g-GT} \) than in \( \text{AST} \). The results of our clinical correlation study are summarized in Table 1.

This \( \text{g-GT} \) method was reliable and simple to perform in the service laboratory. Using equipment normally found in most clinical laboratories, up to 30 analyses per hour may be performed with ease. Following our recommended protocol for establishing methods in the service laboratory,\textsuperscript{17} the \( \text{g-GT} \) assay is now routinely utilized for patient
Table 1
Clinical Correlation Study Results

<table>
<thead>
<tr>
<th>Diagnosis</th>
<th>Number of Patients</th>
<th>Results</th>
<th>% Elevated g-GT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cirrhosis</td>
<td>6</td>
<td>6</td>
<td>100%</td>
</tr>
<tr>
<td>Hepatitis (primary and secondary)</td>
<td>9</td>
<td>9</td>
<td>100%</td>
</tr>
<tr>
<td>Hepatobiliary obstruction, hepatic trauma, acute pancreatitis</td>
<td>6</td>
<td>6</td>
<td>100%</td>
</tr>
<tr>
<td>Cancer of liver and pancreas</td>
<td>5</td>
<td>5</td>
<td>100%</td>
</tr>
<tr>
<td>Cancer metastatic to organs other than liver or pancreas</td>
<td>8</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>3</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Chronic renal failure</td>
<td>16</td>
<td>7</td>
<td>9</td>
</tr>
<tr>
<td>Congestive heart failure</td>
<td>2</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>Myocardial infarct</td>
<td>4</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Pregnancy</td>
<td>2</td>
<td>-</td>
<td>2</td>
</tr>
<tr>
<td>Osteosarcoma and degenerative bone disease</td>
<td>7</td>
<td>0</td>
<td>7</td>
</tr>
<tr>
<td>Miscellaneous diseases with no expected g-GT elevation</td>
<td>17</td>
<td>0</td>
<td>17</td>
</tr>
</tbody>
</table>
care in our hospital. Since its introduction the g-GT assay has been of significant diagnostic value in confirmation of possible hepatobiliary and pancreatic diseases.

**Implementation of Protocol**

Our service laboratory needed a more sensitive and specific test for follow-up on patients with possible hepatobiliary disorders. Additionally, we wanted an assay which would be useful in distinguishing between bone and hepatobiliary disorders. Both g-GT and 5’Nucleotidase assays were considered as likely candidates. The following is a brief description of the format followed during the adoption of our test method.

**Literature Search and Selection of Assay Conditions**

After reviewing the literature concerning g-GT and 5’Nucleotidase, it was determined that a g-GT method would be most useful, due to the inherent phosphate contamination problems associated with 5’Nucleotidase assays. Of the numerous g-GT assay methods presented in the literature, the methods of Szasz, Rosalki and Tarlow, Bondar and Moss, and the recommended method of the Scandinavian Society for Clinical Chemistry and Clinical Physiology seemed to be best suited to the needs of our particular service laboratory.

Preliminary investigation of these selected methods indicated that, with slight modification, the method of Bondar and Moss would meet our needs. The serum volume was decreased to provide a serum to reagent
ratio of approximately 60; the other four methods recommended a ratio of 10 which would cause greater interference from icteric or lipemic sera. The reaction mixture pH was changed to agree with the pH optimum for our substrate reported by Szasz. The reaction temperature was selected at 39°C.

It is worthwhile to comment at this point that we support the concept that standardized "selected" and "reference methods should be considered for adoption in preference to other methodologies whenever these methods are satisfactory to the service laboratory's needs. Two g-GT methods have been proposed as selected or recommended methods by two clinical chemistry societies. Neill and Doggart have recently questioned who, or what society has the authority to declare international selected, recommended, or reference methods? Brown, et al., have proposed definitions of selected, reference, and definitive methods, along with a plea for international cooperation in the designation of these methods; clearly this concept remains in a state of flux. At the present time there is no true reference or definitive method for g-GT analysis according to the criteria proposed by Brown, et al. In view of this, the modified method of Bondar and Moss was selected.

**Determination of Linear Range**

Following our test procedure, serum with an elevated g-GT activity was allowed to react for twenty minutes. The net absorbance change suggested that a serum with an activity of at least 1100 U/liter could be analyzed linearly, as shown in Figure 2. In our clinical correlation study, we found
IlO :-;cra with elevations above 600 U/liter. The conditions of our assay, therefore allowed linear assay of grossly elevated g-GT values, while still permitting adequate sensitivity in the normal range.

**Reagent Blanks**

It was found that the baseline absorbance of each batch of reagent substrate varied as much as 0.050 absorbance units, presumably from uncontrollable acid hydrolysis of the substrate by HCl during the reagent preparation steps. In testing for non-enzymatic substrate degradation during the five-minute reaction time, no absorbance change greater than 0.002 absorbance units took place. However, because non-enzymatic substrate hydrolysis can occur to some degree, we decided that a reagent blank must be included with each test run. Any absorbance change in the reagent blank over the five-minute reaction period must be subtracted from the absorbance change of each sample. Serum blanks are not necessary in this two-point kinetic method.

**Control Sera**

Unassayed Ortho Normal control serum (lot #12M202, Ortho Diagnostics, Inc., Raritan, NJ 08869) was used as the normal control serum. The abnormal control serum should have a g-GT activity that is clearly abnormal, preferably at least an elevation of three times the normal range. No commercial control serum was found that demonstrated a suitably elevated g-GT activity. Therefore, 400 ml of pooled excess human sera was obtained from
hospital patients having elevated liver enzymes. This serum pool was thawed and refrozen five times, centrifuged and filtered. Then after being tested for Hepatitis Associated Antigen it was dispensed into 0.5 ml aliquots and frozen at -20°C, for use as an abnormal control serum pool. An aliquot of each control pool was assayed daily for over two months. The Ortho Normal control sera was found to have a mean g-GT activity of 40 U/liter; the abnormal serum pool had a mean activity of 136 U/liter. Both a normal and an abnormal control were included in each subsequent assay run.

Accuracy

Since enzyme standards are not available, it has been suggested that for enzyme analyses the results should be compared with those from reference methods to determine accuracy. As previously discussed, there is presently no established reference method for g-GT analyses. We agree with Barnett that comparison of two enzyme methods is impractical, especially when there is no reference method available, unless all the assay conditions are exactly the same. Assays performed at differing temperatures or pH will yield differing values. The use of conversion factors to correct for alterations in assay conditions has been challenged. Under these conditions correlation with other g-GT methods was not performed.

Recovery

Recovery was determined by adding 50 ul of abnormal control serum
to sera containing normal g-GT activities. The recovery, after correcting for the increased amount of serum, should be equal to the sum of the two individual activities.

**Precision**

Precision studies for both within-day and day-to-day precision were conducted at two levels of activity utilizing our two control sera pools. After 69 daily assays of both sera pools, the day-to-day precision data was calculated at 95% confidence intervals. The within-day precision data was obtained by analyzing 30 samples each of the two sera pools.

It is worthwhile to comment here that for precision calculations values exceeding $\pm 3$ SD of the mean should be excluded, as they represent error other than random analytical error (technique, contamination, etc.). However, because these "outlier" values represent the same types of errors that patient samples are subject to, they must not be ignored; they should be expressed as a percentage of the total values and presented along with the precision data of the method. Our precision studies showed only a 1% outlier incidence in the normal control pool during the day-to-day precision study. Barnett\(^2\) suggests that methods with outlier frequencies greater than 8% should not be used as they could yield too many dangerous random errors.

**Interfering Factors**

Interference of enzyme activity by bilirubin was checked by adding pure bilirubin standard material to aliquots of several patient sera.
enzyme activities were compared to the respective activities before addition of bilirubin. The effect of hemolysis on g-GT activity was similarly checked. Hemolyzed sera aliquots were prepared by adding a suspension of each patient's own packed red blood cells to the serum and freezing the suspension to induce gross hemolysis.

Veritication of Literature Claims

Considerable controversy exists concerning the effect of glutamate on g-GT activity. In agreement with other investigators, 12,13,18 we were unable to demonstrate the enhancing effect on g-GT activity by free glutamate which was reported by Bondar and Moss.5,6 However, since deleterious effects of glutamate have not been demonstrated, it was included as a precaution in our reaction mixture as recommended by Bondar and Moss.5,6

The literature claims regarding disease correlation with g-GT activities were substantiated during our previously described clinical correlation study.4,7,9,11,15,19,23

Technologist Training

The importance of training technologists to properly perform an assay must not be understated. Regardless of the accuracy, precision, and utility of an assay method, unless the technologist performing the test has been adequately trained and has the necessary expertise in analytical technique, the assay will be, at best, of questionable usefulness as a diagnostic tool.
The test procedure was written following the established format used by our laboratory. Three key technologists representing all working shifts were trained to perform the test and to teach their subordinates the test procedure. Special attention was given to the critical points of the analysis and to potential sources of error. Each technologist was personally "walked-through" the entire assay procedure several times, and was allowed to practice the analysis on selected sera until sufficient expertise was attained to allow the offering of the new test for patient care.

Physician Communication and Solicitation for Utilization

A hospital newsletter was prepared describing the g-GT method, its uses, advantages and disadvantages, reference intervals, price, ordering information, sample requirements, and literature references. This newsletter was distributed to all staff physicians, and the test was discussed by our pathologists during medical rounds.

In addition, a small memo was attached to the outside of each hospital patient's medical chart reminding the physicians of the availability of the new g-GT test. This was performed twice over a one-month period to assure that the information was seen by a large portion of our staff physicians.

Once the new test became routinely utilized, follow-up was begun by contacting the ordering physicians periodically to obtain their opinions as to the clinical usefulness of the test as a diagnostic aid.
Conclusion

We feel that by following our suggested protocol\textsuperscript{17} for establishing a method in the service laboratory that our g-GT method has been properly adopted and implemented in our laboratory. This opinion is fortified by the increasing utilization of the g-GT test for patient care.
Bibliography


IV

SUMMARY

A complete, systematic protocol for the selection, evaluation, and adoption of analytical methodologies in the clinical chemistry service laboratory is critical to improving health care. Such a protocol would permit the standardization of methodology adoption among service laboratories, regardless of geographical location, and would ensure adequate evaluation of analytical methods to ensure optimal reliability and clinical utility.

A complete protocol has been herein presented. It is of significant value to service laboratories in that it offers a standardized, systematic approach to methodology adoption, starting with the assessment of need for a test and continuing through evaluation of the assay’s utility to the physicians.

The significance and utility of the protocol has been tested and verified by the adoption of a γ-Glutamyl Transferase assay method in the Clinical Chemistry Laboratory, University of Utah Medical Center, following the protocol herein presented. Increasing utilization and physician confidence in the assay attests to the validity of the protocol and the entire methodology adoption procedure.
VITA

Name                  Leonard Gary Nielsen
Birthplace           Salt Lake City, Utah
Birthdate            December 29, 1942
High School          Abraham Lincoln High School
                      Denver, Colorado (1959-1961)
Military Service     U. S. Marine Corps (1961-1964)
College              Metropolitan State College
                      Denver, Colorado (1969-1972)
                      B. S. Degree (Summa Cum Laude) in
                      Biological Sciences
Medical Technology   Sisters of Mercy School of Medical
School and Internship Technology, Mercy Hospital,
                      Denver, Colorado (1972-1973)
Registry Certificate American Society of Clinical Pathology,
                       Registered Medical Technologist,
                       MT (ASCP) Registry number 089952
                       (1973)
University           University of Utah
                       Salt Lake City, Utah
                       (1976 - present)
Professional
Organizations       American Society of Clinical Pathology
Professional
Experience          Medical Technologist, Mercy Hospital,
                      Denver, Colorado
                      Chief Medical Technologist/Laboratory
                      Supervisor, Star Valley Hospital,
                      Afton, Wyoming