DERIVATION OF EVALUATION SURVEY DATA FROM PROFICIENCY TESTING FOR INFECTIOUS MONONUCLEOSIS

by

Roger Norris Taylor

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SUPERVISORY COMMITTEE APPROVAL

of a dissertation submitted by

Roger Norris Taylor

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Date

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Chairman, Supervisory Committee

I have read this dissertation and have found it to be of satisfactory quality for a doctoral degree.

19 November 1974
Date

[illegible]
Member, Supervisory [illegible]

I have read this dissertation and have [illegible] to [illegible] quality for a doctoral degree.

19 November 1974
Date

Paul S. Nicholes, Ph.D.
Member, Supervisory Committee

I have read this dissertation and have found it to be of satisfactory quality for a doctoral degree.

19 November 1974
Date

[illegible] Dr. P.H.
Member, Supervisory Committee

I have read this dissertation and have [illegible] it to be of satisfactory quality for a doctoral degree.

Date

Gilbert A. Hill, [illegible]
Member, Supervisory Committee

I have read this dissertation and have found it to be of [illegible] for a doctoral degree.

19 November 1974

Date

J. Lamar [illegible]

Member, Supervisory Committee

I have read this dissertation and hav[illegible] quality for a doctoral degree.

19 November 1974

Date

[illegible] Ph.D.

Member, Supervisory Committee

I have read this dissertation and have found it to be of satisfactory quality for a doctoral degree.

19 November 1974

Date

Lowell A. Woodbury, [illegible]

Member, Supervisory Committee

I have read this dissertation and have found it to be of satisfactory quality for a [illegible]

19 November 1974

Date

[illegible] M.D.

Michael Britt, M.D.

Member, Supervisory Committee

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[illegible]
Date

[illegible], Ph.D.
Chairman, Supervisory Committee

Approved for the Major Department

[illegible], Ph.D.
Chairman/Dean

Approved for the Graduate Council

[illegible], Ph.D.
Dean of the Graduate School

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ABSTRACT

Two standard tests for infectious mononucleosis (the Davidsohn differential and the ox cell hemolysin test) and ten slide tests Monotest (Wampole), Monospot (Ortho), Monosticon (Organon), Diagluto I. M. (Beckman), I. M. Kit (Micro. Res. Corp.), Mono-Diff (Wampole), Bacto-Hetrol (Difco), Monosticon Dri-Dot (Organon), Monophile (BDS) and Rythrotex (BMC) were evaluated as they were used in 50 laboratories within the state of Utah plus two referee laboratories outside the state. The state proficiency testing program for infectious mononucleosis was modified so that the desired evaluation survey data could be obtained. The sensitivity, specificity, reproducibility (within and between shipment) and percent agreement with target results were determined for each test.

Factors which might have affected the test results were evaluated to determine which were correlated with good performance. These factors included: formal education of the technologist performing the test, professional affiliation of the technologist, technologist's experience, technologist's area of major interest, type of laboratory, size of laboratory, number of tests performed, use of the test (screen, confirm or both), technologist's experience with the test and procedural differences.

Since there is a critical need for evaluation survey data in almost every area of clinical laboratory testing and since there are in existence numerous proficiency testing programs in these areas, it is felt that an enormous amount of valuable information could be relatively easily obtained by similar modifications of existing proficiency testing programs.

INTRODUCTION

The primary purpose of proficiency testing is to allow an outside agency to estimate the level of competence that could be expected from a participating laboratory and thereby aid in making decisions concerning the acceptability of the service provided to patients. This objective is obtained by periodic shipment of specimens to be identified or tested for the presence of certain constituents.

The primary purpose of evaluation surveys on the other hand is to obtain statistical data concerning the sensitivity, specificity and reproducibility of the various test procedures being employed. This has been done in the past, e.g., in the syphilis serology evaluation survey conducted by CDC in 1956 (108), by obtaining large numbers of positive and negative specimens and shipping them to a large number of laboratories to be examined. In spite of the critical need for this type of information, evaluation surveys have not been made because of the immense logistical and financial problems involved.

In order to fill the void in the area of evaluation surveying and at the same time circumvent the major problems that have created this void, the hypothesis was suggested that evaluation survey data can be derived from existing

proficiency testing programs with only minor modifications. Volumes of proficiency testing data are generated each year which are of little value in determining the reliability of the tests being employed because variations in the procedures are not identified and the results do not allow statistically significant statements to be made.

A review of the literature reveals that there have been no evaluation surveys made to determine the specificity, sensitivity and reproducibility of the numerous tests and kits used to test for infectious mononucleosis. In fact, in the whole field of serology there is only one area in which critical evaluation surveys have been performed, that is, in syphilis serology; the last such evaluation was carried out in 1956 (47).

Evaluative data are obtained from research laboratories almost exclusively. Comparative results are reported on tests performed in only one laboratory and that is often the laboratory of the author of the test. Much raw data is available in the form of proficiency testing results but such data are inadequate because proficiency testing is designed to determine laboratory performance, not test performance.

An example taken from a preliminary examination of Utah state proficiency testing results for infectious mononucleosis shows some of the possible results and problems (Table I). Application of the chi square (X^2) test to the Monotest (Wampole) results indicates that this test

TABLE I

Preliminary Evaluation of Tests for Infectious Mononucleosis

from Utah State Proficiency Testing Data

	Sensitivity		Specificity		Reproducibility	
	#	%	#	%	#	%
Ox-Cell Hemolysin	9/9	100	6/6	100	5/5	100
Paul-Bunnell	3/3	100	2/2	100	2/2	100
Monotest (Wampole)	9/12	75	8/8	100	8/8	100
I.M. Kit (Micro.Rsh.Corp.)	6/6	100	4/4	100	4/4	100
Monosticon Dri-Dot (Organon)	3/3	100	2/2	100	2/2	100
Monospot (Ortho)	3/3	100	2/2	100	2/2	100
Improved Monotest (Wampole)	3/3	100	2/2	100	2/2	100
Monosticon (Organon)	3/3	100	2/2	100	2/2	100
Total	39/42	92.8	28/28	100	27/27	100

# = # correct/#done
Sensitivity = % of positive samples positive
Specificity = % of negative samples negative
Reproducibility = % of matched samples with identical results

was significantly less sensitive than the other tests ($.01<P<.05$). Differences probably exist between all the tests for the other parameters as well, but the small sample size and poor experimental design do not allow their detection.

We hoped to correct these deficiencies by modifying the proficiency testing program of the Utah State Division of Health so that reliable data could be obtained to evaluate the sensitivity, specificity and reproducibility of the tests for infectious mononucleosis used by the laboratories in Utah.

The significance of this work is that it will allow tests to be evaluated without the enormous expenditure of time and material that has been involved in the past. Because of the cost deterrent most of the tests currently being employed in clinical and public health laboratories have not been investigated to determine their reliability. The original contribution made by this research is that it permits the use of existing programs to provide badly needed information about tests which have not been obtainable by other methods.

Although this research was limited to tests for infectious mononucleosis in Utah, it is anticipated that similar investigations could be conducted on other serologic tests and by other organizations. The adoption of similar procedures by agencies involved in proficiency testing would result in a wealth of valuable information that is not now

available and would correct the present deplorable condition of using unevaluated or underevaluated tests. Clinicians and laboratory personnel would then have reliable information on which to base their judgments concerning the use of laboratory tests.

Evaluation surveys such as this could be used to develop standards for reagents, techniques, and performance like those established for syphilis serology. The benefits of standardization are demonstrated by proficiency testing results. The mean score (percentage of referee laboratory values) for laboratories participating in Utah State's syphilis serology proficiency testing is 98.2 ± 4.9 whereas the mean score for immunology (non-syphilis serology) is 89.0 ± 17.7. This difference is highly significant ($P < .001$) suggestions that with proper evaluation and standardization of non-syphilis serologic tests could markedly improve test performance.

Since the tests in these two areas involve similar procedures, most of the difference must be due to the fact that tests for syphilis have been thoroughly investigated and standardized. The development of such standards for tests on the area of immunology will require an ongoing effort to evaluate new tests, new reagents and new laboratories.

REVIEW OF LITERATURE

Infectious mononucleosis or glandular fever was first described clinically by Pfeiffer in 1889 (81). However, Hoagland (50, 53, 54) does not believe that the disease Pfeiffer described was infectious mononucleosis because of its clinical characteristics and he objects to classifying a disease as a mononucleosis when blood studies were not done. The first case of the disease in the United States was reported to have been observed by West (119) in 1896.

The original description of the disease characterized it as a febrile illness accompanied by sore throat and generalized enlargement of lymph nodes. Türk (107) in 1907 was the first to associate abnormal blood cells with the disease and to categorize the disease as a form of acute lymphatic leukemia.

In 1920 Sprunt and Evans (96) introduced the name infectious mononucleosis and described the unusual cells encountered. In 1923 Downey (35) described the morphological changes in these cells in minute detail.

In 1911 Forssman (43) discovered the immunization of rabbits with sheep erythrocytes caused the production of an antibody which was toxic to guinea pigs and that guinea pig kidney contained an antigen which reacted with this rabbit antibody. He also found that the blood and organs from pigs,

cows, rats, and rabbits contained no antigen of this type which came to be called Forssman antigen. Further studies (2) into the nature of the Forssman antibody revealed that it was but one of a family of heterophile antibodies which have been characterized by Davidsohn and Walker (24) as "antibodies that have the ability to react with antigens that are apparently entirely unrelated to those which stimulated their production." Forssman antigens are widely distributed in nature including group A and B agglutinogens in human blood; in horses, camels, sheep, mice, dogs, cats and chickens; in the Gram negative bacilli of the salmonellae and coliform group; in pneumococci; in toxoplasma and other parasitic organisms; in some fungi; and a variety of hemagglutinogens occuring in viral diseases (110).

Davidsohn in 1929 (22) established the casual relationship between injected horse serum and the elevated agglutinin and hemolysin titers for sheep erythrocytes and applied it to the serological diagnosis of serum sickness. Later he (23) showed that normal control subjects possessed anti-sheep agglutinins of a heterophile nature.

Two years later Paul and Bunnell (80) made a heterophile antibody survey of diseases that they considered to be related to serum sickness. They discovered "quite by accident" that heterophile antibodies were elevated in infectious mononucleosis. This test is entirely nonspecific and is therefore a presumptive test for infectious mononucleosis.

In 1935 Stuart (101), Bailey and Raffel (2), and

Davidsohn and Walker (24) independently differentiated the antibodies for Forssman antigen and those of serum sickness from infectious mononucleosis by the absorption patterns of sheep agglutinins. In 1941 Barrett (3) described the technique of absorption and titration which is now widely used. This differential test has proved to be accurate and specific, although time consuming.

Beer (6) in 1936 reported antibodies in infectious mononucleosis serum for erythrocytes of sheep, beef, goats, horses, pigs, guinea pigs, rabbits and dogs. He found elevated hemolysin titers for beef red blood cells and increased agglutinin titers for sheep, goat and horse cells. The titers for horse erythrocytes were the highest.

Butt and Foord (12) in 1935 developed the first rapid test when they reported that if sera which showed a positive Paul-Bunnell titer (80) were mixed with sheep cells in a hanging drop preparation, agglutination was immediate. Following this observation, Straus (99) developed rapid slide and tube tests. Similar slide screening tests have been used with considerable success by others (77, 85, 112), the numbers of false results being small or absent. The advantage offered by these tests is that they eliminate the need of performing the more laborious tests on a majority of sera.

Gleeson-White et. al. (45) reported that infectious mononucleosis sera agglutinated trypsinized beef erythrocytes in higher dilutions than normal sera. This was confirmed by Tomcsik and Baumann (106).

Wöllner (123) in 1955 developed a differential test for infectious mononucleosis which took advantage of the fact that serum from patients with infectious mononucleosis agglutinates papain treated sheep cells in lower dilutions than untreated sheep cells. Non-infectious mononucleosis serum showed the opposite reaction. The diagnostic value of his test has been confirmed (30, 78, 94).

In 1968 Henle, Henle and Diehl (49) reported the association of antibodies to a herpes-type virus (Epstein-Barr (EB) virus) and infectious mononucleosis. Although the EB virus is suspected of being the etiological agent of infectious mononucleosis this relationship has not yet been conclusively proven. Indirect fluorescent antibody tests using the EB virus antibodies have been developed, but they are not practical from a clinical standpoint because of uncertain specificity of the antibodies and because once a patient develops antibodies, titers remain elevated for years.

Because of the inconvenience of performing the standard tests, a number of rapid slide agglutination tests have been developed over the years (23, 56, 71, 77). The first was developed by Tannen (104) in 1953. These slide tests employ a variety of reagents and techniques and consequently have varying specificities and sensitivities.

In spite of the existence of a number of specific serological tests, a number of authors have emphasized that a complete diagnostic study of a patient suspected of having

infectious mononucleosis should still include clinical manifestations and morphological examination of the blood as well as serological tests (53).

I. SHEEP CELL HETEROPHILE SCREENING TEST (Moloney and Malzone (77)

The sheep cell screening test is a test used in many laboratories as a preliminary test of patient sera to identify those which will require further testing in order to determine if the patient has infectious mononucleosis.

Moloney and Malzone (77) describe a typical sheep cell slide screening test for infectious mononucleosis. According to their procedure, fresh sheep cells were washed three times with a minimum of 10 volumes of normal saline, centrifuged, and the packed cells are stored at 4 C. One drop of the test serum is added to one drop of a freshly prepared 10% suspension of the sheep cells on a glass slide and thoroughly mixed. Any macroscopic agglutination appearing within one minute is accepted as presumptive evidence of infectious mononucleosis and the serum is then tested by a more specific technique.

This test is quite sensitive but it is not very specific. Davidsohn (32) reported a 7.6% incidence of false positive reactions with it.

II. PRESUMPTIVE HETEROPHILE TEST (Paul and Bunnell (80))

Paul and Bunnell (80) first applied the heterophile test to infectious mononucleosis in 1932. This test and

modifications of it have also been published by other investigators (3, 55, 60).

The test consists of heat inactivating the test serum for 30 min at 56 C, then preparing a 1:5 and subsequent two fold dilutions. To 0.25 ml of each serum dilution is added 0.1 ml of 2% sheep erythrocytes which are then mixed and incubated at room temperature for two hours. The tubes are then read for agglutination and the titer reported.

During the active stage of illness, a sheep agglutinin titer of 224 or higher favors the diagnosis of infectious mononucleosis (30). However, the titer of heterophile antibody has not been found to correlate with severity or duration of the disease or with incidence of complications (5).

This test is simple and sensitive but lacks specificity (30, 90). False positive tests have been reported in Hodgkin's disease, sarcoma, polycythemia, agranulocytosis, leukemia and tuberculosis (46, 90). Bunnell (11) maintained that high titers (99) were diagnostic for serum sickness or infectious mononucleosis.

In small laboratories the requirement for maintaining a supply of fresh sheep cells can be inconvenient and expensive.

The original procedure called for overnight refrigeration before reading the titer but later authors have recommended incubation at room temperature (25, 94). Hall (47) has reported that in spite of variations in incubation,

centrifugation, endpoint reaction and other parameters there was no apparent correlation between modifications of the procedure and the results obtained.

III. DIFFERENTIAL ABSORPTION TEST (Davidsohn and Walker (24))

Davidsohn (24, 25, 26) at the same time as others (2, 101), discovered that patients with serum sickness and infectious mononucleosis could be separated from each other and from patients with other heterophile antibodies by differential absorption of their sera with suspensions of guinea pig kidney and boiled beef erythrocytes.

To perform this test, a sample of serum is absorbed with guinea pig kidney and another sample with boiled beef erythrocytes. The titer of agglutinins for sheep erythrocytes is determined for each serum after absorption (30). As defined by Davidsohn et al. (27) the differential test for infectious mononucleosis is positive if the titer of sheep agglutinins after absorption with guinea pig kidney is not more than three tubes lower than the titer of the presumptive test and the sheep agglutinin titer is at least four tubes lower after absorption with beef erythrocytes (72).

Absorption Patterns for Various Heterophile Antibodies (9)

Type of Heterophile Antibody	Absorbed by Guinea Pig Kidney	Absorbed by Beef Erythrocytes
Forssman ("native")	Yes	No or partial
Serum Sickness	Yes	Yes
Infectious Mononucleosis	No or Slight	Yes

A number of investigators (5, 13, 14, 32, 72) have reported using only absorption with guinea pig kidney. They have also reported various criteria for determining positive tests which could be ambiguous when compared with the Davidsohn interpretation. Hall (47) has pointed out that the interpretation of the results of a positive heterophile test is based on the differential absorption pattern which requires the use of both guinea pig (or horse) kidney and beef cell antigens. Beef cell absorption is necessary to distinguish sheep cell antibodies which are not related to infectious mononucleosis. Bender (8) reported a case of Hodgkin's disease which would have been misdiagnosed if beef cell absorptions had not been performed.

The sheep agglutinin differential absorption test is by far the most thoroughly investigated reference test available at the present time (31). On the basis of 600 carefully studied cases, Bender (8) concluded that there is no satisfactory evidence that a positive differential test for infectious mononucleosis can be duplicated by any other condition. In cases with sheep agglutinin titers of 28 or below in the presumptive test, the use of horse cells is recommended (31).

IV. OX CELL HEMOLYSIN TEST (Mason (74))

Elevated beef hemolysin titers in sera of patients with infectious mononucleosis were reported by Bailey and Raffel (2) in 1935, but were not used in a clinical test until 1951. Mason (74) applied the ox cell hemolysin test to the diagnosis of infectious mononucleosis and reported that it was more specific than the classical heterophile antibody tests (6, 25, 80). Since then, many investigators have confirmed the value of this reaction in the serological diagnosis of infectious mononucleosis (30, 37, 69, 76, 82). Peak beef hemolysin titers were found in sera from patients within 2-3 weeks after the onset of illness (30). All sera tested within 6 weeks after onset of illness were still positive. Mason (74) and Leyton (69) also observed that the ox cell hemolysin appeared earlier and remained elevated longer than the sheep cell agglutinins. Lee, et. al., (62) believe that sheep erythrocyte agglutinins and ox cell hemolysins are physically inseparable and imply that tests using these antibodies should have equal reliability.

The procedure used by Cabrera and Carlson (14) and Mikkelsen (76) is as follows: To each of 10 tubes is added 0.5 ml of an 0.85% saline solution containing 0.1 gm of magnesium sulphate per liter. To the first tube, 0.5 ml of inactivated serum is added, and serial dilutions are made. To each tube 0.5 ml of a 1:15 dilution of complement and 0.5 ml of a 2% suspension of washed beef erythrocytes are added. Tubes are shaken and incubated in a 37 C waterbath for 15

minutes. The tubes are then centrifuged at 2000 rev/min for two minutes and compared with a 50% hemolysin standard, which is prepared by adding 0.5 ml of a 2% suspension of beef erythrocytes to 2 ml of distilled water. The titer is the reciprocal of the dilution of the tube which compares most closely to the standard. Titers of 48 or less are considered negative; 96 to 384, suspicious; and 768 or higher, positive.

Hall (47) reports that there are two more commonly used ox cell hemolysin techniques, the CDC method and the Peterson method. The main differences are in the serum diluent, incubation time and significant titer. The Peterson method uses Kolmer saline as a diluent with a 15 min incubation time and a 480 (final serum dilution) significant titers. The CDC method uses Veronal buffer (pH 7.3) as a diluent with a 30 min incubation time and a 40 (initial serum dilution) significant titer. These two titers differ by a factor of 3, i.e., a 1:40 initial dilution becomes a 1:120 final dilution after the complement and ox cells are added. According to Hall's data the CDC method is superior to the Peterson method.

The rationale for using the ox cell hemolysin test for the serologic diagnosis of infectious mononucleosis rather than the heterophile antibody test with absorptions is based on the fact that the ox cell hemolysin is not a naturally occuring antibody (69, 98). Although in infectious mononucleosis a characteristic absorption pattern does occur there are a variety of other conditions in which a similar

pattern may occur (98). Another argument for using the ox cell hemolysin test is that it can be done in one test whereas the Davidsohn technique requires three tests. Some laboratories perform only the sheep cell agglutination or only titration after absorption with guinea pig antigen. With both tests false negative and false positive results may occur. False positive reactions in the ox cell hemolysin test occur rarely. This test generally eliminates the false positive reactions which may occur in sera of patients affected by numerous agents which provoke a rise in non-specific heterophile antibodies (110).

Mason (74) and Peterson et. al. (82) believe that sheep agglutinins and beef hemolysins are similar in the specificity of their reactions, while Leyton (69) thinks that beef hemolysins and sheep agglutinins in infectious mononucleosis serum are different. Lee et. al. (62) were unable to separate beef hemolysins and sheep agglutinins and interpreted this to mean that beef cell hemolysis and sheep cell agglutination are two different manifestations of the same antibody. Their results also indicated that although the two antibodies are physically inseparable they may possess different combining sites responsible for agglutination and hemolysis.

Several other workers (41, 42, 76, 82) have proposed the clinical laboratory use of the ox cell hemolysin test; however, several technical difficulties have been reported. These difficulties include: (1) titers have been reported in terms of final dilution rather than in terms of the serum

dilution; (2) the amount of complement used has varied; (3) the use of fresh complement has produced false positive titers in normal sera; (4) the sera have been diluted with saline containing magnesium sulfate without buffer to control the pH (109).

Hall's (47) comparison of the CDC and Peterson ox cell hemolysin tests show that the CDC method is superior to the Peterson method. Four of the ten laboratories using the Peterson method obtained positive results on two or more of the four positive specimens, while all eleven of the laboratories using the CDC method obtained positive results on two or more positive specimens. These differences (40% vs. 100%) suggest that the CDC method is better. Statistical significance, however, can not be determined because the actual results are not recorded.

V. SHEEP CELL CAPILLARY AGGLUTINATION SCREENING TEST (Lee, Davidsohn and Mih (63))

Lee et. al. (63) developed the capillary screening test for infectious mononucleosis as a result of their observation that almost 80% of the sera submitted to them would not require the use of the entire differential test if a simple screening test were available. They objected to the Paul-Bunnell test because it only eliminated absorption and still required preparation of dilutions. They claim that the advantage of their test over an agglutination test is that as the particulate antigens are allowed to descend through the

small diameter column they come into contact with more antibodies and the chances of aggregation of the particles is enhanced.

One volume of sheep cells are put in a capillary tube followed by 3 volumes of serum being careful that an air bubble does not separate them. The end is then sealed, the capillary is inverted and the cells are observed for agglutination.

The reported 1.3% false negative (3 out of 227) and about 11% false positive (147 out of 1338). These results were confirmed by Bartlet and Castagno (4) when they reported an incidence of false negatives of 3% (3 out of 112) and 11% false positive.

VI. FORMALINIZED HORSE CELL CAPILLARY AGGLUTINATION SCREENING TEST (Sigler (93))

Sigler (93) modified the technique of Lee et. al. (63) by using formalinized horse cells in his capillary screening test. He reported only 0.68% false positive reactions (2 out of 296) and 1.9% false negatives (5 out of 267).

The advantages offered by this test are: stable reagents, serum inactivation is unnecessary, procedure is simple and the test has fewer positives.

VII. ENZYME I TEST (Wöllner (122))

The enzyme I test is based on the observation by Wöllner (122) that serum of patients with infectious mononucleosis agglutinated papain-treated sheep erythrocytes in

lower dilutions than untreated sheep erythrocytes, but the results with normal serum were just the opposite. Inasmuch as this test was positive only 69% of the time he improved it by absorption with treated cells and agglutination with both treated and untreated cells (enzyme II test).

An explanation for the mechanism of operation of the enzyme I test was hypothesized by Wöllner (122). He thought that sheep cells have two types of receptors, one specific for infectious mononucleosis and the other non-specific, and that some of the specific receptors are not readily accessible to antibody. The agglutination titer is the result of the combination of infectious mononucleosis does not contain the specific antibody and consequently has a lower agglutination titer. Papain treatment inactivates the specific receptors and at the same time exposes previously inaccessible nonspecific receptors which results in increased titers in normal serum and decreased titers in infectious mononucleosis serum.

After evaluating the test Davidsohn (30) stated that it furnishes no significant information that is not available with the help of the presumptive test.

VIII. ENZYME II TEST (Wöllner (123, 124, 125))

In the enzyme II test serum absorbed with papain-treated sheep erythrocytes is then titrated with untreated and papain-treated cells. The infectious mononucleosis serum has a sheep cell agglutinatin titer 4 or more tubes higher

with the untreated cells than with the papain-treated cells (30, 123, 124, 125).

It has been proposed that in the enzyme II test the absorption with papain-treated erythrocytes removes the non-specific antibodies from the serum but does not affect the specific antibodies. Testing such serum with untreated sheep cells (having specific receptors) will yield a titer at least 4 dilutions higher than with papain-treated cells (without specific receptors) (125).

Davidsohn et. al. (30) reported that in their laboratory the differential, enzyme II, and beef hemolysin tests yielded generally comparable results.

IX. PAPAIN TREATED SHEEP CELL AGGLUTINATION TEST (Lovric (70))

Papain has been shown to inactivate infectious mononucleosis receptors on sheep erythrocytes (122), but trypsin does not (95). Papain and trypsin do not significantly affect horse agglutinin titers of infectious mononucleosis sera, but both enzymes increase the titers of non-infectious mononucleosis sera (64).

Lovric (70) in 1961 introduced a slide test for infectious mononucleosis using treated and untreated stabilized sheep erythrocytes. This test was based on the earlier agglutination of untreated cells when compared to papain-treated cells by serum from patients with infectious mononucleosis. He later (71) evaluated this test by comparing it to the presumptive and differential tests. No false

positives were reported on 620 patients. An advantage of this slide test is that the cells are reported to be stable for 14 months when stored at 5 C.

X. INDIRECT FLUORESCENT ANTIBODY TEST (48, 89)

In 1968 Henle, Henle and Diehl (49) reported the association of antibodies to a herpes-type virus (Epstein-Barr virus) and infectious mononucleosis. The test is performed by allowing unknown serum to react with a line of EB-3 Burkitt tumor cells smeared on glass slides. The cells have been grown in an arginine-deficient medium to enhance the content of virus. After allowing the serum to react the cells are then washed and fluorescein-labeled anti-gamma globulin is allowed to react with the cells. If antibodies to the virus are present the fluorescein-labeled antibody will bind to them and can be detected with fluorescent microscropy. Although the EB virus is suspected of being the etiological agent of infectious mononucleosis, this hypothesis has not been proven. Indirect fluorescent antibody tests using the EB virus antibodies have been developed but they are not practical from a clinical standpoint because of the uncertain specificity of the antibodies and because once a patient develops antibodies, titers remain elevated for years. Studies have shown (89) that nearly 50% of adults have antibodies which will react with this antigen, thus making the fluorescent antibody test completely useless as a diagnostic tool.

XI. COMMERCIAL SLIDE TESTS AND TEST KITS (33)

As a result of the increasing demand for diagnostic tests and in an attempt to simplify and speed up testing procedures, a number of commercial products are now available for screening and testing for infectious mononucleosis (Table II). Some of the tests are discussed in this work, others have not been discussed because of the lack of availability of literature or the vague and incomplete nature of the available literature.

Hall (47) has reported that commercial slide tests are reliable for detecting moderate to high levels of antibody, but the low or borderline antibody levels can not be detected consistently by them. He also reported a high level of specificity for these tests.

XII. MONOTEST-DENCO TEST (Hoff and Bauer (56))

Stuart et. al. (102) reported that horse erythrocytes contained an antigen which reacted with infectious mononucleosis sera. Beer (6) demonstrated that horse cells reacted in a manner comparable to sheep cells in infectious mononucleosis and serum sickness, but that titers for infectious mononucleosis antibodies were higher when horse cells were used. In 1964 Wilkinson and Carmichael (120) confirmed this observation by demonstrating that in the course of infectious mononucleosis the agglutinin titers in the serum were higher with horse red cells than with sheep red cells. Lee, Davidsohn and Slaby (64) determined that agglutinin titers for horse cells of infectious mononucleosis

TABLE II

List of Test Kits for Clinical Laboratory

(Infectious Mononucleosis)(33)

Manufacturer	Products
BBL (BioQuest)	Reagents for Rapid Plate Screening Heterophile Antibody
BBL (BioQuest)	Differential & OxCell Hemolysin Test
BBL (BioQuest)(Confirmit)	IM Slide Test Kit
Beckman Diagnostics (Diagluto IM)	Rapid Slide Test
Behring Diagnostics	Heterophile Antibody
Bio-Diagnostic Systems (Mono-phile)	Rapid Slide Test
Boehringer Mannheim Cor. (Rythrotex)	Rapid Slide Test
Burroughs Wellcome	Reagents for Agglutination Test
Cappell Laboratories, Inc.	Rapid Slide Test
Colab (Monostat)	Rapid Slide Test
Difco	Agglutination or Differential
Difco (Bacto-Heterol)	Heterol Slide Test
Grand Island Biological Co. (Tekit)	Agglutination Slide Test
Hyland	Reagents for IM Serodiagnosis
Markham Laboratories (Markam Slide Test)	IM Rapid Slide Test
Microbiological Research Corp.	Paper Slide Agglutination Test
Microbiological Research Corp. (IM Kit)	Rapid Slide Test
Organon (Monosticon)	Differential Slide Test
Organon (Monosticon DRI-DOT)	Rapid Slide Test
Organon (Monosticon Quantitative)	Heterophile Antibody Titration
Ortho (Monospot)	Differential Slide Test
Oxford (Heterocyte)	Mirror-Slide Agglutination Test
Rockland (Bi-Mono-Screen)	Slide Agglutination Test
Wampole (Mono-Test)	Slide Agglutination Test
Wampole	Automated Test Materials
Wampole (Mono-Diff)	Differential Slide Test

and non-infectious mononucleosis sera were approximately two dilution tubes (4 times) higher than they were for sheep cells. Barrett (3) had previously observed the persistence of horse cell agglutinins after the loss of sheep cell agglutinins in patients convalescing from infectious mononucleosis. Barrett (3) devised a test for infectious mononucleosis using horse cells in place of sheep cells, but his test was not superior to the sheep cell test.

Cox (18) was the first to use fixed cells and Hoff and Bauer (56) were the first to utilize formalinized horse erythrocytes as the antigen for a rapid slide test for infectious mononucleosis (Mono-Test). As developed by Hoff and Bauer the rapid slide test for infectious mononucleosis consists of mixing one drop of a 4% saline suspension of formalinized horse erythrocytes with one drop of serum (either heated or unheated) on a flat glass slide, mixing at room temperature with a wooden applicator stick, rotating the slide for two minutes and reading for agglutination within this time period using indirect lighting from below over a dark background. Finely granular patterns are read as negative. Positive sera produces coarse agglutination which usually develops within one minute. Saline controls are included.

This test does not require the inactivation of complement and does not require fresh cells. The cells are stable for at least 6 months at 4 C (56). It is sold commercially as the Mono-Test in the United States and as the Denco test

in England (20, 72).

Using the Davidsohn differential test as an arbitrary definition of infectious mononucleosis, Hoff and Bauer (56) reported that the horse cell test gave a positive reaction in 183 cases, while the Davidsohn differential test was positive in 180 cases (98% agreement). Of 80 cases with sheep cell antibodies but no horse cell agglutinins, four were positive for infectious mononucleosis with the Davidsohn differential test (95% agreement) (56). Of 426 cases suspected of infectious mononucleosis the correct diagnosis was reached in 419 (98.5% agreement).

Davidsohn (32) also reported that the horse cell slide test is more specific than the sheep cell screening test and closely approximates the accuracy of the conventional differential absorption test, supporting the claims of Hoff and Bauer. Hoff and Bauer (56) reported one case of serum from a convalescing patient which was positive by the horse cell test but negative by sheep cells. Eighty positive sera with sheep cell titers of 56 or less had negative horse cell tests. A number of other reports have confirmed the high degree of specificity and low incidence of false reactions reported by Hoff and Bauer (20, 32, 72, 83, 92).

Davidsohn (32) reported that the number of false positive reactions encountered with the Mono-Test (0.4%) was considerably less than with the sheep cell screening test (7.6%), but others have reported that it gives about 4% false negative reactions and as many as 15% false positive reactions

when performed in parallel with the heterophile antibody test (88).

Lowell and Kazakaitis (72) found that the Mono-Test gave comparable results to the Paul-Bunnell sheep cell agglutination test with guinea pig kidney absorption. The incidence of false positive results was 0.7%. An incidence of 3.5% false negative tests was found in patients early in the course of their disease and in the convalescent period. They stated that Mon-Test did not become positive earlier than the sheep cell agglutination test (2 cases) and did not persist positively as long as the sheep cell agglutination titer in the recovery phase of the disease (2 cases). These findings are contrary to the findings of Hoff and Bauer (56).

Russell et. al. (88) reported a 4% rate of false positives in 151 negative cases and a 15% rate of false negatives in 51 cases. They also stated that a rare false negative may occur with non-inactivated serum.

Sheil (92) reported no false negatives in 48 positive cases and 8 false positives in 100 negative cases using the Denco test. Dann (20) claimed 99% accuracy for the Denco test in 139 tests. He reported one false positive and one false negative result.

The Mono-Test appears to be more specific than the sheep cell agglutination test in excluding infectious mononucleosis, although not as sensitive in detecting cases with low antibody titers. The Mono-Test is recommended

because of its "specificity, speed, simplicity, stability and low cost" (72).

Carter et. al. (15) use dilutions of patients serum with the Mono-Test in order to report a quantitative titer to the physician. They reported that the Mono-Test titer multiplied by a factor of 56 is equivalent to a presumptive sheep cell titer.

XIII. AUTOMATED MONOTEST (117, 118)

An automated test for infectious mononucleosis has been developed and was recently evaluated by the Connecticut State Department of Health (117, 118). This method makes use of the Autoanalyzer system (Technicon Corp., Tarryton, New York 10591) developed for the Automated Reagin Test (ART) for syphilis (75). Reagents for this test include ART saline and a tanned preserved horse cell antigen. The test can be performed on unheated serum at the rate of 100 samples per hour.

The evaluation of this test involved comparison with the ox cell hemolysin test and the Mono-Test slide test. Of the 991 samples tested 317 were positive for infectious mononucleosis and 674 were negative. The percentage agreement with the clinical diagnosis for the automated, slide and ox cell tests were 95.9, 96.0 and 98.1 respectively. Sensitivity and specificity were not calculated by the authors but were as follows: Automated test - sensitivity 93.7% and specificity 96.9%, Slide test - sensitivity 89.6% and specificity 99.0%, Ox cell test - sensitivity 97.5% and

specificity 98.4%.

They concluded that the ox cell hemolysin test appears to be the most sensitive and specific of the three test procedures, with the slide test the least sensitive and the automated test the least specific. The automated test appeared to be satisfactory as a screening test when reactivity was confirmed by the ox cell hemolysin procedure (117, 118).

XIV. MONO-DIFF TEST (Wampole)

This is a differential absorption slide test which uses horse kidney antigen in place of guinea pig kidney. Horse kidney absorption has been reported to have the advantages of being less expensive, requiring less antigen, exhibiting decreased non-specific absorption and having an end point which is easier to read (29). The indicator cells consist of "preserved, stable" (formalinized) horse erythrocytes as used in the Mono-Test.

There are no data concerning the sensitivity, specificity or reproducibility of this test. The test includes a positive human serum control and the manufacturer recommends using a 0.85% saline negative control.

XV. MONOSPOT TEST (Lee, Davidsohn and Panczyszyn (65))

The spot test (Monospot) is a slide procedure developed by Lee et. al. (65) as a result of their conclusion that citrate preserved horse erythrocytes are superior to formalinized cells in terms of speed and intensity of test

reactions. This test is similar to the Mono-Test except that relatively short-lived citrate preserved horse cells (20% suspension in 3.8% sodium citrate) are used instead of long-time usable formalinized horse cells and the sera are absorbed with guinea pig kidney or beef erythrocytes before addition of the horse cells. It has been reported that unwashed, citrate preserved horse erythrocytes can be stored for 3 months and give stronger and quicker agglutination in infectious mononucleosis than formalinized horse erythrocytes (31).

Wahren (116) reported that the reactivity of the Monospot test was equal to the Paul-Bunnell test, with a sensitivity corresponding to sheep red cell agglutination titers of 20 to 40. One hundred sera from 55 patients with clinically and hematologically verified mononucleosis were seen. Apparent false positive reactions (5-14%) were seen only with sera containing normal or serum heterophile antibodies.

Seitanidis (91) reported that only three out of 210 control sera (1.4%) gave false positive results with the Monospot test when compared with the Paul-Bunnell test. No false negative results were obtained. Rose and Bell (87) believed the Monospot test to be the most accurate slide test, but they considered the Mono-Test to be close in accuracy.

Basson and Sharp (5) found that contrary to the report of Lee et. al. (64) the horse cells as used in the Monospot

test were not more sensitive than sheep cells in detecting borderline positive cases. Davidsohn (31) maintains that if quick results are desirable, the spot test, is in his experience, more dependable both as to sensitivity and specificity than other simplified tests.

The spot test does not quantitate the titer of the specific heterophile antibody as does the Paul-Bunnell reaction. However, this is of little practical importance as the titer of heterophile antibody has not been found to correlate with the severity or duration of infectious mononucleosis or with the complications that may accompany or follow the disease (5).

Evans et. al. (40) suggested that the Monospot test could be used as a confirmatory test for sera giving a positive sheep cell screening test. They claimed that agreement between these two tests could be considered diagnostic for infectious mononucleosis and would eliminate the necessity of Paul-Bunnell titrations in about 86% of the sera. Most tests could be reported 24 hours earlier and would not require titration.

Wolf et. al. (121) reported two cases of false positive Monospot tests which occurred in malignant lymphoma patients. False positive reactions have also been observed in hepatitis (5, 116).

"If further experience confirms the high diagnostic accuracy so far achieved with the horse cell test, it may be assumed that differential absorption techniques will

become largely, if not completely, unnecessary for the routine diagnosis of infectious mononucleosis." (32)

XVI. UNABSORBED SPOT TEST (Cabrera and Carlson (14))

Lee, Davidsohn and Panczyszyn (65) designed experiments using preserved horse erythrocytes and treating them with enzymes and alcohols in an attempt to eliminate the absorption process but concluded that it could not be eliminated. They were satisfied to eliminate the centrifugation process by using a fine suspension of antigen which would not obscure the agglutination.

Cabera and Carlson (14) developed the unabsorbed spot test for infectious mononucleosis by decreasing the hematocrit of the horse erythrocytes used in the spot test from 20% to 7 or 8%. By so doing it became unnecessary to absorb the serum with guinea pig kidney antigen. They claimed that this simplified the test without altering its specificity or sensitivity. The commercially preserved horse erythrocytes remained in good condition from four to five months in the refrigerator. For use the cells were diluted in a ratio of five parts of cells to six parts of 3.8% sodium citrate.

The test is performed by placing a drop of saline and a drop of inactivated serum on an oval-marked slide with a white background. These are then mixed and a drop of horse erythrocytes (hematocrit 8%) are added. This suspension is mixed well with a wooden applicator stick, left undisturbed

for 30 seconds and read for agglutination (14).

The developers claim that the unabsorbed spot test is as sensitive and specific as the spot test and is simpler to perform.

XVII. BACTO-HETROL SLIDE TEST (Difco)

Although the literature provided by the manufacturer (34) does not specifically state what the test reagents are or how they are prepared, it implies that it is Lane's (61) modification of Lovric's (70) modification of Brumfitt and O'Grady's (10) enzyme differential slide test. The Bacto-Hetrol Reagent P which is described as being "a stabilized erythrocyte suspension for routinely screening sera for heterophile antibodies as a presumptive test for infectious mononucleosis" is apparently a suspension of formalinized or citrated erythrocytes. The Bacto-Hetrol Reagent C which is described as "a stabilized erythrocyte suspension used to confirm a positive heterophile test as being that of infectious mononucleosis" is probably a suspension of enzyme treated sheep erythrocytes. The test includes both positive and negative human serum controls.

No data are available as to sensitivity and specificity.

XVIII. MONO-PHILE (Bio-Diagnostic Systems, Inc.)

This test utilizes "specially treated horse erythrocytes, color-enhanced" as the indicator cells and includes guinea pig kidney absorption by means of guinea pig kidney extract on the disposable test card. The test includes positive and

negative controls prepared from human serum.

No data are available concerning sensitivity and specificity.

XIX. RYTHROTEX (Boehringer Mannheim Corp.)

This test utilized formalinized horse erythrocytes as the indicator cells (19) but adds polystyrene latex particles which the manufacturer claims eliminates the problem of hemolysis of the indicator cells and difficulty in resuspending the cells. The instruction sheet accompanying the test claims that this system does not react with Forssman antibodies or agglutinate with those antibodies associated with serum sickness. No literature is cited concerning the results of evaluation of this test. The test kit includes positive and negative controls prepared from human serum.

XX. MONOSTICON (Organon)

Monosticon is a differential slide test which employs a "specially prepared stable suspension of processed sheep erythrocyte" which have been dyed blue and includes absorption with both guinea pig and beef antigens. A positive animal serum control is included in the kit.

The test is intended to give a positive result on sera which have a Davidsohn guinea pig absorption heterophile titer of 56 or above, but no data are available to document actual specificity or sensitivity.

XXI. MONOSTICON DRI-DOT (Organon)

The Monosticon Dri-Dot test uses horse erythrocytes (dyed) as indicator cells and includes absorption with guinea pig antigen only. Both cells are dried on the test card and are mixed with the serum, plasma or blood in steps (guinea pig antigen first).

The test is intended to give a positive result on sera which have a guinea pig absorbed Davidsohn sheep cell titer of 28 to 56, but data are not available to document sensitivity or specificity of this test.

XXII. I. M. KIT (Microbiological Research Corp.)

According to the information submitted to the Utah State Division of Health by the author laboratory, this test utilizes formalinized horse erythrocytes and does not involve an absorption step. Any agglutination is considered positive. The test kit provides both positive and negative controls prepared from human serum. No data are available on sensitivity or specificity.

XXIII. DIAGLUTO I.M. (Beckman)

Diagluto I. M. is a slide test which utilizes a "proprietary stabilized horse-cell substance" as an indicator and includes beef and guinea pig antigen absorption. The kit includes a positive human control serum and the manufacturer recommends the use of saline as a negative control. The test can be used as either a screening test with no absorption or as a confirmatory test with the absorptions.

Literature contains no reference to documentation of sensitivity or specificity.

MATERIALS AND METHODS

I. RATIONALE BEHIND APPROACH

In the syphilis serology evaluation survey performed by the Center for Disease Control (CDC) (108), large numbers of specimens were obtained from subjects selected according to exposure to the disease or other conditions which could give positive results. Sera from these individuals were placed in small tubes, numbered, and sent to laboratories participating in the study. This is an almost ideal method for performing an evaluation survey but it is rarely used due to the enormous costs involved and the number of personnel and institutions required.

As an alternative to this prohibitively expensive procedure, it is proposed that state, federal and private proficiency testing programs be used to derive evaluation survey data. This approach necessitates some deviation from ideal conditions. However, the project is designed to compensate for such compromise and critically needed information can be obtained at much less cost.

In order to use proficiency testing data to differentiate the various tests to be evaluated, emphasis must be placed on the borderline or grey areas and on possible interfering conditions. Thus, paired specimens with titers in these areas or containing possible interfering substances

were included.

Since the number of samples ordinarily used in proficiency testing is limited, therefore, random sampling of patients can not be relied upon. Such a procedure would be unlikely to yield significant results. To obtain significant results the specimens must of necessity be artificially produced to simulate the conditions desired.

Detailed information concerning the exact procedure used by each laboratory was obtained in order to evaluate the effect of various modifications on the performance of the test. To expidite obtaining this information, we sent a predominately multiple choice questionnaire specifically designed for these tests to each laboratory. Background information about the laboratory and the technologist was obtained by a similar method.

During the year, each shipment of test sera was used as proficiency testing unknowns. The results were graded, tabulated and then reported to the participating laboratories along with the usual critiques and suggestions. At the end of the year the results were combined and the necessary evaluation parameters were calculated.

Results were grouped by the type of tests used and were subgrouped by modifications of the standard test procedure used. The combined data were used to calculate specificity, sensitivity, and reproducibility of each of the groups and subgroups. The presence, if any, of statistical difference among the tests for each of the parameters was determined

by analysis of variance and application of Duncan's new multiple mean test.

It has been shown (108) that there is a proportionate, inverse relationship between sensitivity and specificity in serologic tests for syphilis, and preliminary studies using CDC (47) proficiency testing data (Table III and Figure I) indicate that this is also true of tests for infectious mononucleosis. On this basis correlation coefficients were calculated for these two parameters.

Sensitivity was determined by calculating the percentage of positive specimens reported to be positive; specificity was determined by calculating the percentage of negative specimens reported to be negative; and reproducibility was determined by calculating the percentage of identical results obtained on paired specimens. Within and between shipment reproducibility were determined as well as overall reproducibility. Agreement was determined by calculating the percentage of results identical to the target values.

Factors which might affect the reliability of a test were measured by segregating and tabulating the test results by parameters and such variables as laboratory size, technologist training and experience, technologist experience with the particular test, and then determining if there was a significant difference by analysis of variance of Duncan's test.

Data processing and computer programming was performed

by Dr. Donna Olsen of the Department of Family and Community Medicine at the University of Utah using the facilities of the University of Utah Computer Center.

II. RECRUITING PARTICIPANT LABORATORIES

There are about 75 laboratories in the state of Utah and 66 laboratories are involved in the state's laboratory improvement program. These include the laboratories enrolled in the various proficiency testing programs as well as reference laboratories in this and other states. Of these laboratories over 40 participate in either state or College of American Pathologists (CAP) proficiency testing for infectious mononucleosis. It was anticipated that at least 50 laboratories would participate.

Letters were sent to each laboratory soliciting their cooperation (Appendix A). They were requested to indicate which tests they performed and would be willing to have evaluated, and were asked to fill out the appropriate Background Questionnaire (Appendix B) and Test Procedure form (Appendix C). Changing tests during the evaluation was discouraged. As an incentive to participate in this program, laboratories within the state were given credit for satisfaction of proficiency testing requirements for state approval. Also, the participating laboratories were to be identified in publications resulting from this study. Laboratories were coded as they are in state proficiency testing and results were tabulated by code numbers. Each

TABLE III

Preliminary Correlation of Sensitivity and Specificity

in Tests for Infectious Mononucleosis *

	Sensitivity		Specificity	
	#	%	#	%
Beckman	58/60	96.6	14/15	93.3
Difco	27/28	96.4	7/7	100.0
Organon	125/132	94.7	31/33	93.9
Ortho (Monospot)	394/428	92.1	103/107	96.3
Wampole (Monotest)	293/328	89.3	82/82	100.0
Other	35/40	87.5	6/10	60.0
Total	932/1016	91.7	243/254	95.7

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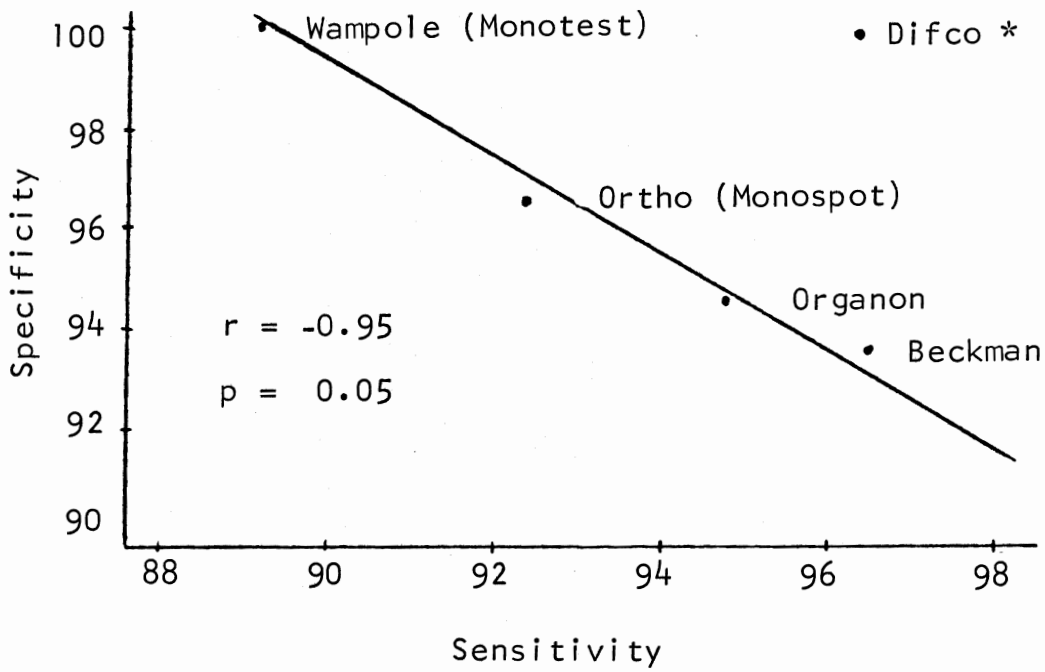
* Data taken from CDC proficiency test results (47)

FIGURE I

Preliminary Correlation of Sensitivity and Specificity in Tests for Infectious Mononucleosis **



* Difco data not included in calculating correlation coefficient because of the small sample size.

** Data taken from CDC proficiency test results (47).

laboratory's code number was known only to the State Division of Health and the individual laboratory. A list of participant laboratories was published but their code numbers were not identified. This procedure was used to encourage participation and at the same time eliminate the fear of being identified as a poor performer on these tests. As an additional incentive, copies of the project report were sent to each laboratory.

By reference to Utah state and CAP proficiency testing results, it was determined that the distribution of tests for infectious mononucleosis used in Utah could be expected to be approximately as seen in Table IV. This indicated that good comparisons could be expected from the first three tests, but that the others might or might not have enough users to give significant results.

The questionnaires were used to determine pertinent background information and details concerning the performance of the test, which in turn was used to determine under what conditions the different tests could be expected to give satisfactory results and what factors could be correlated with good performance. The questionnaires were designed to obtain the necessary data as efficiently and accurately as possible because it was recognized that excessive demands on the laboratories would be a deterrent to their participation.

TABLE IV

Expected Distribution of Tests for Infectious Mononucleosis in Utah

Test	Manufacturer	%
Monotest	Wampole	32
Monospot	Ortho	17
Monosticon	Organon	17
Ox-Cell Hemolysin		7
Presumptive (Paul-Bunnell)		5
Differential (Davidsohn)		5
Diagluto I.M.	Beckman Diagnostics	5
I.M. Kit	Micro. Research Corp.	5
Other		7
		100%

III. PREPARATION OF UNKNOWNS

Since maximum titers of heterophile antibodies usually occur during the second or third week of illness, and may persist for 4 to 8 weeks, this allowed ample time to obtain serum from positive patients screened at the State Division of Health. Physicians of patients with high heterophile titers were contacted and their cooperation was elicited in obtaining blood or serum samples.

If all 75 laboratories in the state were to participate in this project, and 4 ml of serum with a titer of 224 were required for making positive unknowns for each laboratory, then 300 ml of serum with a titer of 224 or an equivalent volume-titer combination would be sufficient. Since sera with titers of 1792 or above are frequently seen, only 38 ml or less of such serum would be enough to prepare all of the positives needed for this project. To provide serum for negative samples and diluent for positive samples, 1500 ml of negative serum would be required.

Serum was used as positive only if the patient was determined to have infectious mononucleosis by serologic, hematologic and clinical criteria. "Most authorities agree that the three essential criteria for confirming a diagnosis of infectious mononucleosis are as follows:

1. A clinical picture characterized by sore throat, exudative pharyngotonsillitis, fever, generalized malaise, and lymph node enlargement. Splenomegaly is present in a majority of the cases and hepatitis may occur, but there is

rarely marked clinical jaundice.

2. That heterophile antibodies in the serum of patients with infectious mononucleosis can be detected and/or measured serologically.

3. A characteristic blood picture (110)."

Bender (8) and Hoagland (51) have emphasized the importance of adhering to these criteria, and how deviation from these criteria has created considerable confusion in the literature.

Positive serum was collected on 12 December 1973 from a patient who met these three diagnostic criteria. Onset of illness had occurred on about 1 November 1973 and was characterized by the conditions listed in Table V and Figures II and III.

Ideal test specimens would be those which duplicate the characteristics of clinical test specimens as nearly as possible. In this program, routine proficiency testing specimens were used but were selected in such a way as to provide as much test evaluation data as possible. Proficiency testing for immunology in Utah consists of four shipments of 5 specimens each, but it was necessary to increase this to 6 specimens per shipment for this project in order to obtain all the necessary data.

The 24 specimens consisted of 12 sera (3 pools) positive for infectious mononucleosis and 12 negatives (3 pools). Equal numbers of positive and negative sera were selected in

TABLE V

Clinical, Hematologic and Serologic Characteristics of Disease in Patient Used as Source of Positive Serum

---

Clinical

Lymphadenopathy
Pharyngitis
Splenomegaly
Hepatomegaly
Prostration

Hematologic (performed 15 Dec. 1973)

White Count 12,000 cells/mm^3

Schilling Differential (31)

40% Segmented Neutrophilic Granulocytes (2 or more lobed nuclei)
2% Band Cells (Neutrophils with a single lobed nucleus)

45% Lymphocytes (50% Atypical and 5% Downey forms)

2% Blasts
7% Monocytes
2% Eosinophils
1% Basophils
1% Plasmocytes

Serologic

	29 Nov 1973	12 Dec 1973
Presumptive titer	229,376	896
G.P.K. Absorbed titer	28,672	448
B.E. Absorbed titer	14	< 7
Ox Cell Hemolysin titer (CDC Method)	--	5120

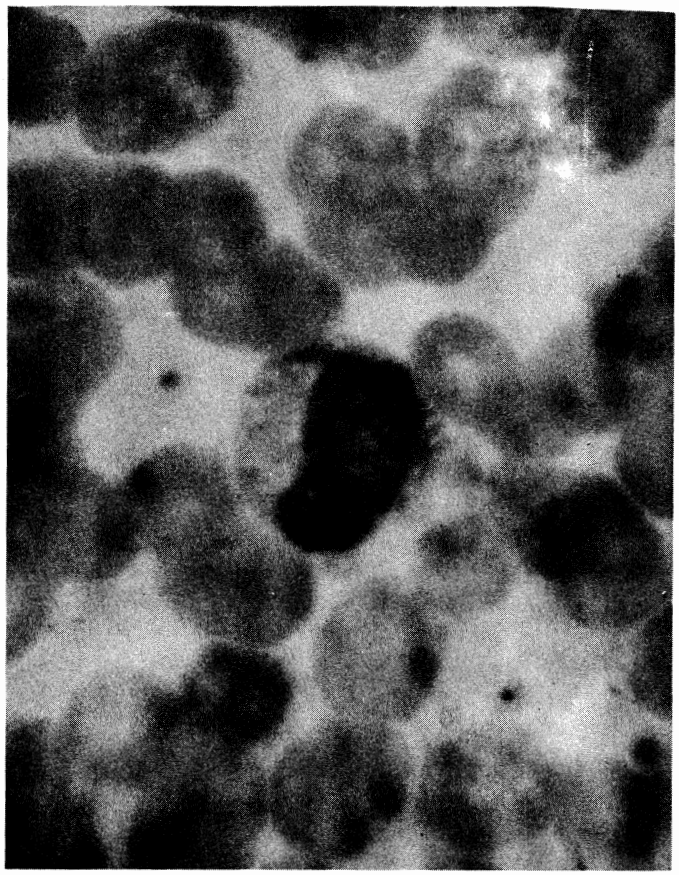


FIGURE 11

Characteristic large atypical (mononuclear) lymphocyte in which the eccentric nucleus is oval, kidney-shaped.

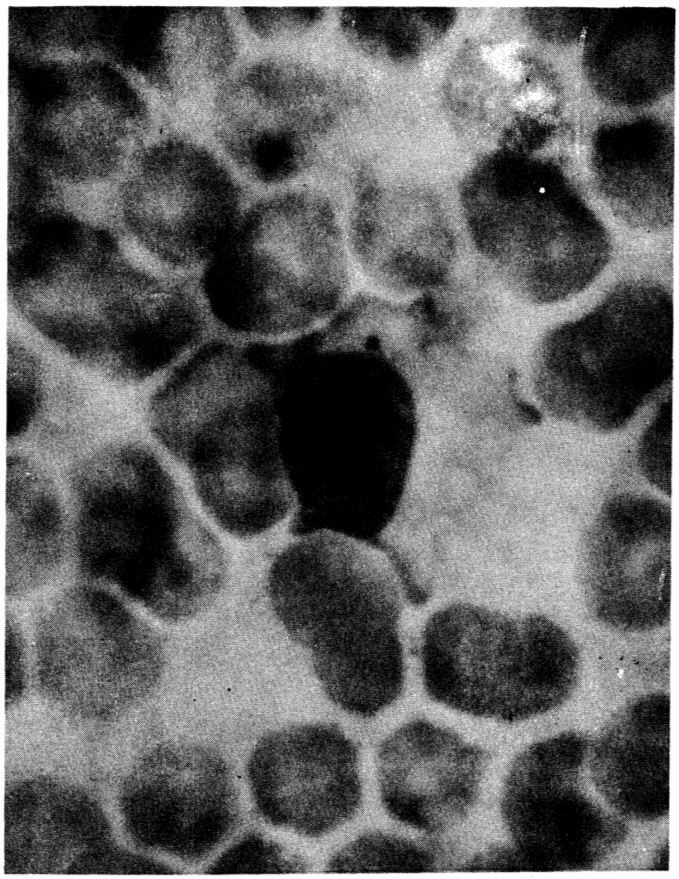


FIGURE III

Atypical lymphocyte with irregular (scalloped) cell margins and pseudopodia-like projections.

order to give equal numbers of tests for calculating both sensitivity and specificity. All the specimens were paired so that as much data on reproducibility as possible could be obtained. This design resulted in 12 tests for each of the parameters to be measured, i.e., specificity, sensitivity and reproducibility.

Since CDC proficiency testing data for infectious mononucleosis test procedure suggests that the critical area is on sera with presumptive heterophile titers of 56 to 224, the positive specimens were in this range. The specimens consisted of 3 quadruplets (within and between shipment pairs) of 2 fold dilutions of a pool with a titer of 224. This resulted in titers of 56, 112, and 224. The samples were tested for ox cell hemolysins to verify that these titers were in the expected relationship to the heterophile titers. This insured that the ox cell hemolysin test would have the same level of sensitivity and specificity that it would be expected to have on specimens normally encountered in the clinical situation.

This procedure gave a maximum number of quadruplets within the critical range and at the same time the quadruplets have a known relationship to each other. An additional advantage of this technique is that titration tests can be used as a measure of reproducibility because multiplying the resultant titers by the dilution factors should result in identical titers.

Inasmuch as the negative specimens were to be used to

measure specificity, they should include sera which could possibly result in false positives. The Davidsohn differential test, and some kit tests, differentiate between infectious mononucleosis and serum sickness or heterophile antibodies; therefore, inclusion of sera exhibiting these properties in the negative group would be desirable if available. Davidsohn (31) states that the only false positives encountered in his thirty plus years of experience with infectious mononucleosis were in patients with rheumatoid arthritis or Hodgkins's disease. False positive ox cell hemolysin tests have been reported (47) in patients that have received group O blood with group specific substances added. If sera from such patients could have been obtained it would have been included.

A procedure used by CDC (47) in their proficiency testing is to add a small amount of rabbit anti-sheep erythrocyte serum (hemolysin) to negative sera. These agglutinins will not be completely absorbed by guinea pig or horse kidney antigen nor by beef cell antigens. This component was included in one pool of the negatives. It is recognized that these do not reflect negatives as encountered in clinical practice but are designed to detect conditions which could give false reactions with the necessarily limited number of specimens used in proficiency testing.

Freezing of specimens was chosen over lyophilization because of the additional sources of error introduced by the latter procedure. Lyophilization would require accurate

dispensing and reconstituting of the sample with the added variable of the purity and sterility of the water used. Since these are not sources of error in testing routine specimens, it was decided to use the freezing technique.

The ox cell hemolysin titer of the positive serum (12 Dec 1973) was determined to be 5120. The test was run with Beckman Complement Lot #310009 and diluent Lot #310805; fresh ox cells obtained from the Midvale Packing Company, 420 South Main, Midvale, Utah; and freshly prepared veronal buffer. Known positive and negative controls were run in parallel. Titration was repeated at a later date with identical results. The heterophile presumptive titer of this positive serum was determined to be 896 with a borderline reaction in the tube with a 1:1792 dilution.

Pools were prepared to have titers of 56 and 112 by diluting 15.6 ml and 31.2 ml respectively of the positive serum (titer 896) to a total of 250 ml with previously tested negative serum. These pools were checks to verify their ox cell hemolysin and heterophile agglutination titers with the following results:

	Ox-cell Titer	H.A. Titer
"56 Titer" pool	160	112
"112 Titer" pool	640	448
Undiluted Positive	5120	896

The "224 Titer" pool was made by adding 0.3 ml of positive serum (29 November 1973) with a titer of 229,376 as

determined by the routine titration of the originally submitted serum, to 250 ml of negative serum.

Negative pools N_1 and N_2 were simply negative sera. Negative pool N_3 was adjusted to a titer of 56 with rabbit anti-sheep hemolysin by the following procedure:

Two ml of BBL Anti-sheep Hemolysin Lot #304924 (reconstituted with Lot #305679 diluent) with a heterophile agglutination titer of 560 plus 25 ml of rabbit anti-sheep hemolysin (titer 512) which was obtained from Dr. Stanley Marcus of the University of Utah were combined and brought to a total volume of 272 ml with previously tested heterophile negative serum.

All of these pools were tested twice for heterophile agglutination titer and once for ox cell hemolysin titer, with the results shown in Table VI. Obviously the high titer serum was not as high as the routine titration had indicated. To correct this error 20 ml of "112 Titer" pool (acutal titer 448) was added to the "224 Titer" pool which resulted in 270 ml of serum with a titer of 56 and was marked P_1. Since the "56 Titer" pool actually had a titer of 224, it was used as the pool for that titer and was marked P_3. The other positive serum was obtained by combining 75 ml of the "112 Titer" pool (actual titer 448) with 225 ml of negative serum which resulted in 300 ml of serum with a titer of 112 and was marked P_2. All of the positive sera were rechecked several times to verify that they were the proper titer.

TABLE VI

Titration of Serum Pools

Pools	1st HA Titer	2nd HA Titer	Ox Cell Titer
$Negative_1$	< 7	< 7	< 10
$Negative_2$	< 7	< 7	< 10
Negative with Hemolysin	56	56	< 10
"56 Titer"	224	224	640
"112 Titer"	≧ 224	448	1280
"224 Titer"	7	14	20

All sera were sterilized by Seitz filtration directly into aspiration flasks. The serum was then dispensed into 2 ml pink stoppered vacutainer tubes by means of a special, sterilized apparatus. This apparatus consisted of a hose which could be attached to the spout of the aspirator flask; a needle for insertion into the vacutainer tubes; a bell shaped glass cover over the needle to prevent bacterial contamination; and a clamp on the hose to control the flow of serum.

The vials were labeled and frozen at -70 C. Samples of each pool were then thawed and tested to determine the effect of freezing on the titers. There were no changes.

Samples were shipped by packing the vacutainer tubes in Blood Mailers #339 (Polyfoam Packers, Chicago, Ill. 60645) and enclosing these mailers in Human Blood Mailing Envelopes. Instruction Sheets (Appendix H) and Test Report Forms (Appendix G were sent to all participants. Shipments were made through the Division of Health mail room and were all sent First Class.

Table VII shows the specimen numbers and the pools of serum which were used in each specimen. The P indicates a positive serum and the subscript indicates the pool of positive serum used. The N indicates a negative or possible false positive serum.

The six quadruplets were arranged so that there are two within shipment pairs and two between shipment pairs for each quadruplet. This allows the measurement of within

TABLE VII

Distribution of Pools Among Shipments

1st Shipment Spec #	Pool	2nd Shipment Spec #	Pool	3rd Shipment Spec #	Pool	4th Shipment Spec #	Pool
SB-1-74	N_2	SB-7-74	N_2	SB-13-74	N_1	SB-19-74	N_3
SB-2-74	N_2	SB-8-74	P_1	SB-14-74	N_3	SB-20-74	N_1
SB-3-74	P_1	SB-9-74	N_2	SB-15-74	N_1	SB-21-74	N_1
SB-4-74	P_1	SB-10-74	P_1	SB-16-74	P_2	SB-22-74	P_2
SB-5-74	P_3	SB-11-74	P_3	SB-17-74	P_2	SB-23-74	P_2
SB-6-74	P_3	SB-12-74	P_3	SB-18-74	N_3	SB-24-74	N_3

Pool	Computer #	Titer
P_1	1	56
P_2	2	112
P_3	3	224
N_1	4	Neg
N_2	5	Neg
N_3	6	Neg (Hemolysin)

shipment reproducibility, between shipment reproducibility and overall reproducibility. The specimens were assigned numbers by using a combination of balanced design and randomization. After the balanced design was determined the actual pools to be included in each shipment and the numbers to be assigned to each were determined by reference to a table of random digits.

Specimens were prepared and shipped as routine proficiency testing specimens. Four quarterly shipments of 6 specimens were made. Instructions and Test Report forms (Appendices D and E) accompanied each shipment.

The test procedures used in testing and preparation of the specimens were as follows:

A. Paul-Bunnell Presumptive Test Procedure (31,60, 80, 126).

1. Heat inactivate 1 ml of test serum and 1 ml of positive control serum of known reactivity for 30 min at 56 C.
2. Set up 2 sets of 13 tubes (11x25mm) in a rack. Place 0.4 ml of 0.85% saline in the first tube and 0.25 ml in each of the remaining tubes.
3. Add 0.1 ml of the inactivated serum to the first tube, mix and transfer 0.25 ml to the second tube, and so on until the twelfth tube is reached. Discard 0.25 ml from the twelfth tube. The serum dilutions are 1:5, 1:10, 1:20, etc.
4. Add 0.1 ml of 2% sheep red blood cell suspension to each tube, including the thirteenth, which is the control. The final dilutions are 1:7, 1:14, 1:28, etc.. Shake each tube

to mix.

5. Incubate at room temperature for 2 hr.

6. Results are read after shaking the test tubes to resuspend the sediment. Check with the naked eye. If no clumping is visible, place the tube horizontally on the stage of the microscope and read with a low power objective.

7. Titer is the reciprocal of the highest serum dilution still showing agglutination.

8. Controls must react properly in order for the test results to be reliable. The 13th tube should show no agglutination and the positive control should be within 2 standard deviations of its known titer.

B. Davidsohn Differential Test Procedure (9, 31, 60, 126)

1. Place in a test tube (85 x 13 mm) 1 ml of boiled guinea pig kidney antigen and in another test tube 1 ml of boiled beef erythrocyte antigen.

2. Add 0.2 ml of heat inactivated serum (56 C for 30 min).

3. Shake and incubate at room temperature for 3 min.

4. Centrifuge at 1500 rev/min for 10 min or longer until supernatant fluid is clear.

5. Remove the supernatant fluid with a capillary pipette, making sure not to pick up particles.

6. Set up as many tubes (10 x 75 mm) as needed, according to the titer of the presumptive test. Add 0.25 ml of physiologic saline to each tube except the first.

7. Add 0.25 ml of absorbed serum to the first and second tubes.

8. Mix the second tube and transfer 0.25 ml to the third tube, etc.. Discard 0.25 ml from the last tube. The serum dilutions are 1:5, 1:10, 1:20, etc..

9. Add 0.1 ml of 2% sheep red blood cell suspension to each tube. Shake well. Final dilutions are 1:7, 1:14, 1:28, etc..

10. Incubate at room temperature for 2 hr.

11. Results are read after shaking the test tubes to re-suspend the sediment. Check with the naked eye. If no clumping is visible, place the tube horizontally on the stage of the microscope and read with a low power objective.

12. Titer is the reciprocal of the highest serum dilution still showing agglutination.

13. Test is positive for infectious mononucleosis if:

    A. Titer of guinea pig kidney absorbed serum is not more than three dilutions or tubes lower than the titer of the presumptive test, and

    B. Titer of beef erythrocyte absorbed serum is at least four dilutions or tubes lower than the presumptive titer.

14. A positive control of known titer should be run in parallel with each batch of tests and control titers should be within 2 standard deviations of known titer to verify reliability of test procedure. If test is run at a different

time than the presumptive or with a different batch of cells or saline, a cell control should be included and should produce no agglutination.

C. Ox Cell Hemolysin Test Procedure (CDC Method) (109, 110)

1. Make a 1:10 dilution of test sera (0.3 ml serum plus 2.7 ml buffer) in Veronal buffered saline and incubate at 56 C for 30 min.
2. Place ten 12 x 75 mm test tubes in a test tube rack for each specimen plus one tube for the ox cell control and one for the complement-ox cell control.
3. Add 0.5 ml of Veronal buffered saline to the second through the tenth tube in each row. Add one ml to the ox cell control tube and 0.5 ml to the complement-ox cell control tube.
4. Add 0.5 ml of the 1:10 dilution of inactivated serum to the first and second tubes of the appropriate row. Beginning with the second tube (1:20 dilution), mix and transfer 0.5 ml to the third tube and continue through the tenth tube. In order to have identical volumes, discard 0.5 ml from the last tube.
5. Prepare a 50% hemolytic endpoint standard by adding the following reagents, in the order listed, to a 12 x 75 mm test tube:

A. 0.25 ml of a 2% ox cell suspension in Veronal buffered saline.

B. 1.0 ml of Veronal buffered water.

C. 0.25 ml of diluted (1:15) complement.

6. Add 0.5 ml of a 2% ox cell suspension to each tube.

7. Add 0.5 ml of a 1:15 dilution of complement to each tube except the ox cell control tube.

8. Shake the tubes to mix the contents.

9. Incubate the test and control tubes at 37 C for 30 min.

10. Centrifuge the test and control tubes at 150 G for two minutes.

11. Read and record results. The tube which most nearly matches the 50% hemolysis standard by visual inspection is considered the serum titer and point.

12. Controls must react properly for test to be reliable. Ox cell control and complement-ox cell control must now show any hemolysis. The positive control should be within 2 standard deviations of its known titer.

13. In absence of horse serum injections, a titer of 40 or above is significant in 95% of patients.

D. Veronal Buffered Saline (109, 110)

1. Stock buffer solution (5 X concentrated)

a. Combine the following in a 2 liter volumetric flask in the order listed:

Distilled water	1500 ml
NaCl	83.00 gm
Na-5,5diethylbarbiturate	10.19 gm
1 N Hydrochloric Acid	34.58 ml

Magnesium-Calcium solution containing 1 M $MgCl_2$ and 0.3 M $CaCl_2$ (20.33 gm $MgCl_2 \cdot 6H_2O$ and 4.4 gm $CaCl_2 \cdot 2H_2O$ in 100 ml distilled water 5.00 ml

b. Fill to the 2.0 liter mark with distilled water. Mix thoroughly.

c. Check the pH of the stock buffer before refrigeration by making a 1:5 dilution with distilled water. The pH of the diluted stock must be 7.3 to 7.4. If the pH is not in this range, discard and prepare fresh stock buffer. Make sure pH of water used is near 7.

2. Gelatin-water solution.

a. Add 1.0 gm of gelatin to 100 ml of distilled water. Bring to boil to insure solution of gelatin.

b. Add 700 ml of distilled water at room temperature.

c. Chill in refrigerator. (This solution should not be held longer than 1 week to avoid contamination.)

3. Working Veronal buffer solution.

a. Dilute stock buffer solution 1:5 with gelatin-water solution. Store in refrigerator. Working solution should not be stored longer than 24 hr. The pH must be 7.3 to 7.4.

E. Veronal Buffered Water (109, 110)

1. Stock Veronal buffered water.

a. Combine reagents in a 1 liter volumetric flask in the order listed:

Distilled water	300-400 ml
Na-5,5diethylbarbiturate	5.1 gm
1 N Hydrochloric Acid	17.25 ml

b. Fill to the 1.0 liter mark with distilled water.

2. Working Veronal buffered water.

a. Dilute stock buffer 1:5 with distilled water.

F. 2% Ox or Sheep Red Blood Cell Suspension (109, 110)

1. Preparing and washing cells.

a. Pipette 10 ml of fresh beef or sheep blood (collected in equal volume of Alsever's solution) through a gauze filter into a 15 ml graduated centrifuge tube. Fill tube with cold Veronal buffered saline and centrifuge at 600 G (2000 rev/min with 13 cm radius or 1700 rev/min with 19 cm radius) for 5 min.

b. Carefully remove supernatant fluid and the white cell layer by suction without disturbing the erythrocytes.

c. Fill centrifuge tube again with cold Veronal buffered saline. Thoroughly resuspend the cells by gently mixing with a pipette. Centrifuge at 600 G for 5 min, and repeat the process for a total of three washings. If the supernatant fluid is not colorless after the second washing, cells are too fragile and must not be used.

d. Resuspend the cells once more in cold Veronal buffered saline and centrifuge for 10 min at 600 G

to pack the cells.

e. Record the volume of packed cells in the centrifuge tube and remove the supernatant fluid. Care should be taken to remove as much fluid as possible without disturbing the cells.

2. Standardization of 2% cell suspension by centrifugation (109, 110).

a. Prepare a 2% cell suspension by adding 1 ml of packed red cells to 49 ml of Veronal buffered saline.

b. To check the density of the 2% cell suspension, pipette 10.0 ml into a 12 or 15 ml graduated centrifuge tube and centrifuge at 600 G for 10 min. A 10.0 ml aliquot of a properly prepared cell suspension should produce 0.2 ml of packed cells. NOTE: The accuracy of the graduated centrifuge tube should be predetermined.

c. When the volume of packed cells is under or above the 0.2 ml point, the cell suspension should be adjusted. The quantity of Veronal buffered saline which must be added to or removed from the cell suspension is determined by the following formula:

Corrected volume of cell suspension =

$$\frac{\text{Actual reading of centrifuge tube}}{\text{Correct reading of centrifuge tube}} \times \text{Volume of cell suspension}$$

d. Keep the cell suspension in the refrigerator when not in use. Always shake the flask gently before

use to secure an even suspension of the erythrocytes which settle on standing. NOTE: Cells may also be standardized by using the centrifuge method described for the Laboratory Branch Complement Fixation (LBCF) technique (109, 111). In the Sanborn-Fromer cell counter, this suspension contains $25 \times 10^4 \pm 2 \times 10^4$ red cells per mm^3.

3. Standardization of 2% cell suspension by spectrophotometer (109).

a. Prepare a 2% cell suspension by adding 1 ml of packed red cells to 49 ml of Veronal buffered saline.

b. Pipette 2.5 ml of this suspension into a 15 ml volumetric flask and fill to the mark with Veronal buffered water. Allow to stand for 10 min to be sure lysis is complete.

c. Read the optical density (O.D.) of the lysed suspension in a 12 X 75 mm cuvette using a Coleman Jr. Spectrophotometer Model 6-D set at a wave length of 545 lambda.

d. The target value of a 2% suspension (diluted 1:6) in this instrument is an optical density of 0.556 ± 0.020.

e. Correct the optical density by adding or removing buffer from the original suspension. The required final volume of the suspension is determined by the following formula:

Corrected volume of cell suspension =

$$\text{Volume of cell suspension} \times \frac{\text{O.D. obtained}}{0.556}$$

f. Keep the cell suspension in the refrigerator when not in use.

G. Reconstitution of Lyophilized Complement (C')

1. Allow the diluent and the lyophilized complement vials to come to room temperature.
2. Remove the crimps from the vials carefully and slowly to insure that the lyophilized complement does not escape.
3. Pipette the diluent slowly into the complement. Do not splash. To reconstitute 7 ml vials, pipette 5 ml of diluent. To reconstitute 20 ml vials pipette 15 ml of diluent. (0.75 ml diluent per ml of original serum).
4. Allow the complement to stand at room temperature until all the large clumps have dissolved. This usually takes 1 to 2 hr.
5. Place the complement in a 4 C refrigerator overnight.
6. Prepare a 1:15 dilution in cold Veronal buffered saline for use in the ox cell hemolysin test.

H. Alsever's Solution (58)

Glucose	24.6 gm
Na citrate (dihydrate)	9.6 gm
NaCl	5.04 gm
Distilled water	1200 ml

Adjust to pH 6.1 with critic acid and sterilize by

filtration.

I. Guinea Pig Kidney and Beef Erythrocyte Suspensions (9)

1. 20% Guinea Pig Kidney Suspension.
   a. Keep guinea pig kidneys frozen until used.
   b. Thaw guinea pig kidneys.
   c. Wash in physiological saline until washings are free of blood.
   d. Mash into fine pulp.
   e. Make a 20% suspension of this pulp in saline.
   f. Boil in water bath for 1 hr.
   g. Correct the loss of water by adding water.
   h. Add phenol to a concentration of 0.5%. When stored in refrigerator this suspension keeps for many months.
2. 20% Beef Erythrocyte Suspension
   a. Wash beef erythrocytes three times with physiological saline.
   b. Pack well by centrifugation.
   c. Resuspend in saline to make a 20% suspension.
   d. Boil the suspension in water bath for 1 hr.
   e. Make up loss of water by adding water.
   f. Add phenol to a concentration of 0.5%.
   g. Store in refrigerator.

IV. ANALYSIS OF RESULTS

As quarterly results were obtained they were evaluated and appropriate modifications in the program were made as

necessary. When all the data had been collected it was segregated by test procedure and the sensitivity, specificity, reproducibility and agreement with target values were calculated for each test. Reproducibility was subdivided into intra-shipment and inter-shipment reproducibility.

Within shipment reproducibility was calculated by comparing the results obtained on each of the paired samples within the shipment that were from the same pool. Pairs with identical results were given a value of one, i.e., positive-positive, weak positive-weak positive, negative-negative. Pairs with opposite results, i.e., positive-negative and negative-positive, were given a value of zero; and pairs differing by half steps, i.e., positive-weak positive or weak positive-negative, were given a value of one half. Within shipment reproducibility is the total of the values given to each pair divided by the number of pairs, expressed as a percentage.

Between shipment reproducibility was calculated in a similar manner except that since there was no rational basis for pairing the between shipment samples from the same pool, values were obtained for all the possible combinations and the total was divided by the total number of possible combinations and the result was expressed as a percentage. Since for each pool there were two samples in the first shipment and two samples in the second shipment, there were four possible combinations of pairs for between shipment reproducibility for each pool.

Total reproducibility was calculated by totaling the values of all of the possible within and between shipment pairs and then dividing by the number of pairs and expressing the result as a percentage. If all four specimens from one pool were tested this resulted in six possible combinations. This method of calculating total reproducibility was chosen over the method of averaging the within and between shipment reproducibilities in order to eliminate differences in total score resulting from distribution of results among the specimens. In other words, this system was chosen because it gives identical scores on results which differ only in the location of the result. For example, if the results of the four samples from the same pool had been positive-positive on the first shipment, and negative-negative on the second shipment, the within shipment reproducibility would have been 100% (2/2), the between shipment reproducibility would have been 0% (0/4), and the reproducibility would have been 33% (2/6). If the same results had been obtained but with different locations, e.g., positive-negative on the first shipment and positive-negative on the second shipment, the within shipment reproducibility would have been 0% (0/2), the between shipment reproducibility would have been 50% (2/4). In both cases, two specimens were positive and two were negative, and the total reproducibility was 33%. If the averaging method had been used the total reproducibility would have been 50% in the first example and 25% in the second.

Sensitivity was determined by calculating the percentage of positive specimens reported to be positive; specificity was determined by calculating the percentage of negative specimens reported to be negative; and agreement was determined by calculating the percentage of results identical to the target values.

EXPERIMENTAL RESULTS

Due to changes within laboratories, additional information and oversights in the preparation of the questionnaire it was necessary to change some of the codes on the questionnaires. On all three forms (Appendices B, C and E) the codes for type of test were changed to the following:

1. Bacto-Hetrol (Difco)
2. Davidsohn Differential
3. Monotest (Wampole)
4. Monospot (Ortho)
5. Monosticon (Organon)
6. Ox Cell Hemolysin
7. Diagluto I. M. (Beckman)
8. I. M. Kit (Micro. Research Corp.)
9. Monosticon Dri-Dot (Organon)
10. Mono-Diff (Wampole)
11. Monophile (Bio-Diagnostic Systems)
12. Rythrotex (BMC)

On the Background Questionnaire (Appendix B) the following changes were made:

Registration was changed to read:

1. A.S.C.P. (American Society of Clinical Pathologists)
2. A.M.T. (American Medical Technologists)
3. I.S.C.L.T. (International Society of Clinical

Laboratory Technologists)

4. None

Area of Major Interest was changed to read:

1. Chemistry
2. Bacteriology
3. Serology
4. Hematology
5. Blood Banking
6. Research
7. General Medical Technology

Type of laboratory was changed to read:

1. Private Hospital
2. Government Hospital (City, County, State or Federal)
3. Clinic
4. Independent Laboratory
5. Independent Research Laboratory
6. Military Laboratory
7. Church Hospital
8. Public Health Laboratories

On the Test Procedure form (Appendix C) the type of indicator cells was changed to read:

1. Sheep (fresh)
2. Horse (formalinized)
3. Horse (citrated)
4. Beef (fresh)
5. Other
6. Sheep ("processed")

7. Horse with polystyrene latex

Absorption was changed to read:

1. None
2. Guinea Pig Kidney
3. Beef Erythrocyte
4. G.P.K. and B.E.
5. Other
6. Horse kidney and B.E.

On the Test Report form (Appendix E) the type of controls used was changed to read:

1. Positive
2. Negative
3. Positive and Negative
4. None

Table VIII shows the results of this evaluation broken down by type of test. Monotest (Wampole) was by far the most frequently used test in this evaluation with Monospot (Ortho), Monosticon (Organon), I.M. Kit (Micro. Res. Corp.), Diagluto I. M. (Beckman) and Monosticon Dri-Dot (Organon) following in the order listed.

Of the tests used by three or more laboratories, the most sensitive was Monosticon Dri-Dot (Organon) and the least sensitive was the Ox Cell Hemolysin. The Davidsohn Differential and Diagluto I. M. (Beckman) both achieved 100% specificity while Monosticon (Organon) and Monosticon Dri-Dot (Organon) received the lowest values for specificity.

Within shipment reproducibility was consistently about

TABLE VIII

Evaluation of Results by Type of Test

Test	No.*	% of total	Sensitivity	Specificity	Reproducibility			Agreement
					Within	Between	Total	
Bacto-Hetrol (Difco)	2	2.3	100.0	100.0	100.0	100.0	100.0	100.0
Davidsohn Differential	4	4.6	91.8	100.0	96.0	92.0	96.0	96.0
Monotest (Wampole)	27	30.7	90.7	96.3	99.0	89.4	94.6	93.0
Monospot (Ortho)	13	14.8	95.5	94.2	97.5	91.2	95.6	94.3
Monosticon (Organon)	10	11.4	92.6	87.0	96.3	91.4	95.9	88.3
Ox-Cell Hemolysin	4	4.6	68.8	95.8	100.0	83.5	89.0	79.2
Diagluto (Beckman)	8	9.1	84.4	100.0	97.9	94.3	96.5	90.6
I.M. Kit (MicroResCorp)	10	11.4	91.7	96.7	95.1	91.6	93.9	94.2
Dri-Dot (Organon)	7	8.0	100.0	86.6	96.0	89.5	94.7	93.3
Mono-Diff (Wampole)	1	1.1	100.0	100.0	100.0	---	100.0	100.0
Monophile (Bio-Diagnostic Systems)	1	1.1	100.0	100.0	100.0	---	100.0	100.0
Rythrotex (BMC)	1	1.1	100.0	100.0	100.0	---	100.0	100.0
Total	88	100.0	91.5	94.9	97.6	90.4	95.1	92.4
F Probability			.110	.466	.551	.086	.631	.425

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used).

5 percentage points higher than the between shipment reproducibility ($P < .001$).

The Davidsohn differential obtained the best agreement with target values but it was not significantly better than the slide tests. Part of the reason for the poor performance by the ox cell hemolysin test can be attributed to the fact that one of the three laboratories changed procedures during the survey and another changed personnel during the survey. This coupled with the fact that even though the Peterson method gave positive titers on positive specimens these titers were not above the minimum significant level established by the author of the test.

There were no significant differences between any of the parameters for any of the tests evaluated. Figure IV shows the correlation between sensitivity and specificity. There was an inverse relationship with $r = -0.70$, which is significant ($P < .02$). The results were analysed by the t test for dependent variables which estimates the probability that the null hypothesis (H_o $r \neq 0$) is valid.

Table IX shows the distribution of tests by quarter and reflects changes in usage. Two laboratories changed from Monotest (Wampole) to Monospot (Ortho) and following changes were made by one laboratory each: Monotest (Wampole) to Monophile (Bio-Diag. Sys.), Monotest (Wampole) to Mono-Diff (Wampole), Bacto-Hetrol (Difco) to Diagluto I. M. (Beckman), Monosticon (Organon) to Monospot (Ortho) and Monospot (Ortho) to Monosticon (Organon). One laboratory

FIGURE IV

Correlation of Sensitivity and Specificity

in Tests for Infectious Mononucleosis

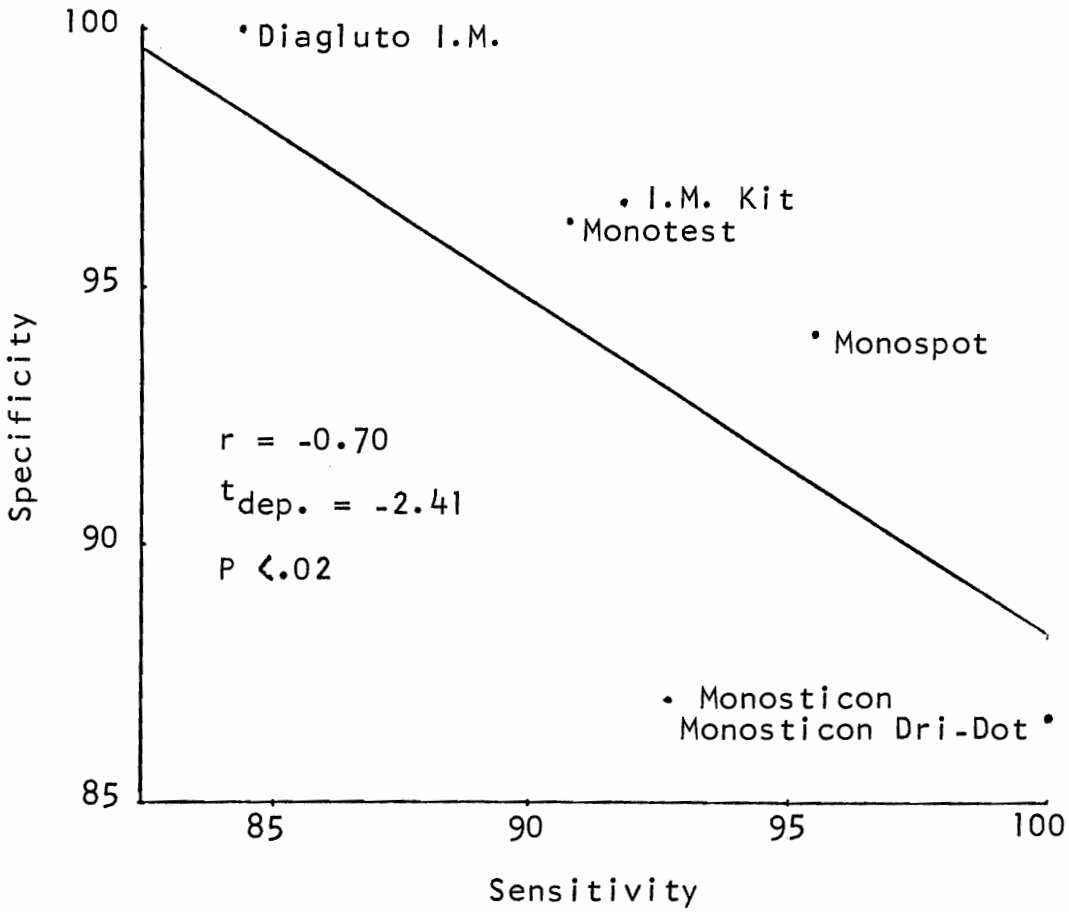


TABLE IX

Distribution of Tests by Shipment

Test	1st Shipment	%	2nd Shipment	%	3rd Shipment	%	4th Shipment	%	Total	%
Bacto-Hetrol (Difco)	1	1.8	1	1.8	2	3.9	1	1.8	2	2.3
Davidsohn Differential	2	3.7	3	5.6	3	5.9	3	5.5	4	4.6
Monotest (Wampole)	21	38.9	19	35.2	17	33.3	17	30.9	27	30.7
Monospot (Ortho)	6	11.1	6	11.1	9	17.6	7	12.7	13	14.8
Monosticon (Organon)	8	14.8	6	11.1	4	7.8	5	9.1	10	11.4
Ox-Cell Hemolysin	3	5.6	3	5.6	2	3.9	2	3.6	4	4.6
Diagluto (Beckman)	4	7.4	4	7.4	4	7.8	5	9.1	8	9.1
I.M. Kit (MicroResCorp)	5	9.3	8	14.8	6	11.8	7	12.7	10	11.4
Dri-Dot (Organon)	4	7.4	4	7.4	4	7.8	5	9.1	7	8.0
Mono-Diff (Wampole)	0	0	0	0	0	0	1	1.8	1	1.1
Monophile (Bio-Diagnostic Systems)	0	0	0	0	0	0	1	1.8	1	1.1
Rythrotex (BMC)	0	0	0	0	0	0	1	1.8	1	1.1
Total	54	100	54	100	51	100	55	100	88	100

used the ox cell hemolysin test throughout but alternated between Monospot (Ortho) and I. M. Kit (Micro. Res. Corp.) as the screening test. Another laboratory started using Monospot (Ortho) and Monosticon (Organon) then changed to Monospot (Ortho) and the Davidsohn differential then to Davidsohn differential and Monotest (Wampole), and finally to the Davidsohn differential and Rythrotex (BMC). Still another laboratory started the evaluation using Monospot (Ortho), changed to Monosticon (Organon) and I. M. Kit (Micro. Res. Corp.) and finally Monospot (Ortho) and I. M. Kit (Micro. Res. Corp.). In spite of these changes noted over 80% of the laboratories did not change tests during this evaluation.

Table X shows the percentage agreement with target values by quarterly shipment and shows the rise in average during the year. The third and fourth quarter overall results were significantly higher (5 percentage points) than the first and second quarter results ($P < .05$) as determined by "student's" t test ($t = 2.47$, d.f. = 211). Presumably this is attributable to increased proficiency which resulted from proficiency testing.

Table XI shows the evaluation of results by type of laboratory. In this table two military hospital laboratories were included in the government hospital group and research laboratories were included in the independent laboratories group. Private hospitals achieved significantly lower ($P < .01$) sensitivity (69.0% vs. 93.7%) than government

TABLE X

Percentage Agreement by Shipment

Test	1st Shipment	2nd Shipment	3rd Shipment	4th Shipment	Total
Bacto-Hetrol (Difco)	100.0	100.0	100.0	100.0	100.0
Davidsohn Differential	83.5	88.7	100.0	100.0	96.0
Monotest (Wampole)	90.6	89.5	97.1	97.1	93.0
Monospot (Ortho)	97.2	91.7	90.8	100.0	94.3
Monosticon (Organon)	95.9	94.5	79.2	86.6	88.3
Ox-Cell Hemolysin	66.7	67.0	100.0	83.5	79.2
Diagluto (Beckman)	79.2	91.8	100.0	100.0	90,6
I.M. Kit (MicroResCorp)	90.0	91.6	100.0	92.9	92.2
Dri-Dot (Organon)	100.0	100.0	83.5	93.4	93.3
Mono-Diff (Wampole)	---	---	---	100.0	100.0
Monophile (Bio-Diagnostic Systems)	---	---	---	100.0	100.0
Rythrotex (BMC)	---	---	---	100.0	100.0
Average	90.5	90.4	94.5	95.7	92.4
F Probability	.309	.594	.134	.631	.475

TABLE XI

Evaluation of Results by Laboratory Type

	Number*	Percent of total	Sensitivity	Specificity	Reproducibility Within	Reproducibility Between	Reproducibility Total	Agreement
Private Hospital	4	4.5	69.0	100.0	99.0	96.0	97.5	82.3
Government Hospital	25	28.4	95.3	89.6	97.4	89.6	94.7	91.2
Clinic	12	13.6	93.8	99.3	97.7	90.3	95.5	96.6
Independent Lab	12	13.6	95.2	94.0	98.7	87.4	93.5	94.7
Military	4	4.5	87.5	100.0	100.0	100.0	100.0	91.8
Church	28	31.8	91.1	96.1	96.9	89.5	94.8	93.4
Public Health	3	3.4	75.0	100.0	97.3	97.3	97.3	85.0
Total	88	100.0	91.5	94.9	97.6	90.4	95.1	92.5
F Probability			.009	.200	.636	.686	.687	.251

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used).

hospitals, clinics or independent laboratories. Private hospitals and public health laboratories had significantly lower sensitivity ($P < .05$) than government hospitals and independent laboratories (69.0% & 75.0% vs. 95.2% & 95.3% respectively). Private hospitals also achieved significantly lower ($P < .05$) agreement than clinics (82.2% vs. 96.6%). There were no other significant differences in performance between laboratories in this study but there may have been if the number of laboratories evaluated had been larger. Many of the differences were substantial but due to small sample size they are not significant. The reason for the poor performance by the private hospitals is not known, but it may be that in an effort to obtain profits the quality of testing is compromised. It is possible that if the most economical tests, control procedures and personnel were employed and this attitude was carried to an extreme in the laboratory, the quality of testing could be adversely affected.

Table XII shows the evaluation of results by level of education. Technologists with a baccalaureate degree achieved slightly better agreement than did either the high school graduate or the technologists with masters degrees (92.8% vs. 90.0% and 89.8% respectively), but the differences were not significant. The one "technologist" with a doctorate did very well (100% sensitivity, specificity and agreement) but not significantly better than any other group. Here again larger sample size may have revealed a

TABLE XII

Evaluation of Results by Level of Education

	Number*	Percent of total	Sensitivity	Specificity	Reproducibility Within	Reproducibility Between	Reproducibility Total	Agreement
High School	10	11.4	85.9	95.9	98.0	94.2	96.9	90.0
B.S.	71	80.7	92.4	94.5	97.0	90.8	95.3	92.8
M.S.	5	5.7	86.6	96.6	99.2	82.6	88.2	89.8
Ph.D.	2	2.3	100.0	100.0	100.0	92.0	97.0	100.0
Total	88	100.0	91.5	94.9	97.6	90.4	95.1	92.5
F Probability			.410	.517	.683	.392	.121	.601

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used).

significant difference.

Table XIII shows the evaluation of results by professional affiliation of the technologists. The two I.S.C.L.T. registered technologists were about 10 percentage points lower in agreement than the other three groups (79.5% vs. 92.8%) but this difference was not statistically significant. At the .05 probability level the I.S.C.L.T. technologists achieved significantly lower sensitivity than the other two professional association's members. And at the .01 probability level the I.S.C.L.T. technologists achieved significantly lower sensitivity than the A.M.T. registered technologists. In other words the I.S.C.L.T. registered technologist were more likely to report a negative result on a positive specimen than technologists with other affiliation or non-registered technologists.

Table XIV shows the evaluation of results by amount of experience of the technologist. Though none of the differences were significant the results obtained by the groups with zero to ten years of experience were generally better than the results for the group with more than ten years experience.

Table XV shows the evaluation of results by amount of experience with the particular test being used. Sensitivity was significantly lower ($P < .05$) for the group with three or more years of experience with the test employed than for the other two groups (85.2% vs. 93.9%). Reproducibility within the shipment was significantly lower ($P < .05$) for

TABLE XIII

Evaluation of Results by Professional Affiliation of the Technologist

Professional Affiliation	No.*	Percent of total	Sensitivity	Specificity	Reproducibility Within	Reproducibility Between	Reproducibility Total	Agreement
A.S.C.P.	62	70.4	92.9	95.1	97.2	90.0	94.9	93.6
A.M.T.	5	5.7	100.0	81.2	96.0	97.3	97.4	89.6
I.S.C.L.T.	2	2.3	66.5	100.0	100.0	91.5	94.5	79.5
None	19	21.6	87.3	97.4	99.2	89.4	95.4	91.0
Total	88	100.0	91.5	94.9	97.6	90.4	95.1	92.5
F Probability			.020	.058	.298	.686	.555	.214

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used).

TABLE XIV

Evaluation of Results by Amount of Experience of the Technologist

Experience in years	Number*	Percent of total	Sensitivity	Specificity	Reproducibility Within	Reproducibility Between	Reproducibility Total	Agreement
0 - 2	26	29.5	92.9	93.9	98.6	91.0	96.4	92.7
3 - 10	49	55.7	91.3	96.2	97.6	89.8	95.0	93.2
>10	13	14.8	89.2	91.8	96.0	90.9	93.1	89.5
Average	88	100.0	91.5	94.9	97.6	90.4	95.1	92.5
F Probability			.689	.460	.255	.394	.388	.561

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used).

TABLE XV

Evaluation of Results by Amount of Experience with the Test Employed

Experience with test in years	Number*	Percent of total	Sensitivity	Specificity	Reproducibility Within	Reproducibility Between	Reproducibility Total	Agreement
0 - 1	46	52.3	93.3	95.7	98.1	91.2	96.3	93.6
2 - 3	18	20.4	95.4	93.4	95.3	89.6	92.8	94.5
> 3	24	27.3	85.2	94.6	98.5	89.9	94.5	88.9
Total	88	100.0	91.5	94.9	97.6	90.4	95.1	92.5
F Probability			.038	.682	.044	.512	.175	.147

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used).

the group with two years of experience with the test than in the other two groups (95.3% vs. 98.2%). This suggests that evaluations of technologist's performance needs to be an ongoing effort and should not be discontinued after minimum qualifications have been achieved. Also, it seems likely that continued interest in the tests being performed is an important factor in maintaining proficiency.

Table XVI shows the evaluation of results by technologist's area of major interest. Agreement was significantly lower ($P < .05$) for technologists claiming general medical technology as their area of major interest than for technologists whose area of major interest was hematology or blood banking (86.4% vs. 96.5% and 95.5% respectively). Also, specificity was significantly lower ($P < .05$) for the general medical technologist than it was for the chemists (88.0% vs. 100%). In general, technologists specializing in hematology or blood banking obtained the best results; with bacteriologists and serologists next and chemists and general medical technologists obtaining the poorest results.

Table XVII shows the evaluation of results by the type of controls used. Since 96% of the tests were performed with at least a positive control the effect of the use of controls on the reliability of the test could not be determined in this study.

Table XVIII shows the evaluation of results by purpose of test use. Most laboratories (52.4%) used the same test as both a screening and confirmatory test. Many laboratories

TABLE XVI

Evaluation of Results by Technologist's area of Major Interest

Area of major interest	No.*	Percent of total	Sensitivity	Specificity	Reproducibility Within	Reproducibility Between	Reproducibility Total	Agreement
Chemistry	12	13.6	84.7	100.0	97.9	90.2	95.6	90.4
Bacteriology	23	26.1	92.8	95.7	97.4	87.9	93.8	94.0
Serology	9	10.2	91.8	97.6	97.3	91.5	94.8	94.3
Hematology	13	14.8	96.8	96.2	97.8	89.9	95.3	96.5
Blood Bank	12	13.6	96.6	95.8	99.0	95.8	98.5	95.5
General	19	21.6	87.3	88.0	97.0	90.6	94.3	86.4
Total	88	100.0	91.5	94.9	97.6	90.4	95.1	92.5
F Probability			.191	.118	.509	.577	.567	.070

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used).

TABLE XVII

Evaluation of Results by Type of Controls Used

Controls Used	Number*	Percent of total	Sensitivity	Specificity	Reproducibility Within	Between	Total	Agreement
Positive	33	16.6	86.4	95.4	94.4	---	94.4	89.4
Negative	5	2.5	90.0	90.0	93.3	---	93.3	93.4
Pos & Neg	158	79.4	85.8	95.7	97.5	---	97.5	93.0
None	3	1.5	100.0	100.0	100.0	---	100.0	100.0
Total	199	100	86.2	95.6	96.9	---	96.9	92.5
F probability			.688	.675	.269	---	.269	.463

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used).

TABLE XVIII

Evaluation of Results by Purpose of Test Use

Use of test	Number*	Percent of total	Sensitivity	Specificity	Reproducibility Within	Reproducibility Between	Reproducibility Total	Agreement
Screen	34	41.5	90.4	94.8	97.8	91.6	95.5	91.9
Confirm	5	6.1	91.8	100.0	95,2	89.0	94.0	96.0
Both	43	52.4	92.5	95.0	97.6	90.0	95.0	93.2
Total	82	100.0	91.6	95.2	97.6	90.7	95.1	92.8
F Probability			.650	.636	.510	.604	.542	.667

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used).

(41.5%) use their tests for screening only, with the remainder (6.1%) designating a particular test as a confirmatory test. Agreement and specificity were a little better for those tests which were used as confirmatory test only, but not significantly so.

Table XIX shows the evaluation of results by technologist's confidence in the test. Most laboratories (79.8%) used tests in which the technologist had "good" confidence and none of the laboratories used tests in which the technologists had "poor" confidence. Although none of the differences were significant the tests in which technologists had only "fair" confidence performed slightly better in general than did the tests in which the technologist expressed "good" confidence. It may be that some skepticism about the reliability of the test could result in better performance by the test.

Table XX shows the evaluation of results by type of indicator cells used. Agreement was significantly lower ($P < .05$) for fresh beef cells than it was for fresh sheep cells, or the three groups of horse cells (79.2% vs. 97.3% and 93.5% respectively). The beef cells were significantly less sensitive ($P < .01$) than any of the other cell types (68.8% vs. 92.6%). These differences are probably not due to inadequacy of the beef cells but to problems encountered during the evaluation, such as small number of laboratories using the beef cell tests, changes in personnel and procedures in those few laboratories and the high titer specified

TABLE XIX

Evaluation of Results by Technologists Confidence in the Test

Technologist's Confidence	Number*	Percent of total	Sensitivity	Specificity	Reproducibility Within	Reproducibility Between	Reproducibility Total	Agreement
Good	63	79.8	90.6	94.7	97.7	90.7	95.1	91.8
Fair	16	20.2	93.8	95.3	96.9	90.8	94.8	94.3
Poor	0	---	---	---	---	---	---	---
Total	79	100	91.3	94.8	97.5	90.7	95.1	92.3
F probability			.468	.647	.549	.411	.646	.431

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used).

TABLE XX

Evaluation of Results by Type of Indicator Cells Used

Indicator cells	Number*	Percent of total	Sensitivity	Specificity	Reproducibility Within	Reproducibility Between	Reproducibility Total	Agreement
Sheep (fresh)	6	6.8	94.5	100.0	97.3	96.0	97.3	97.3
Horse (formalinized)	46	52.3	90.0	97.1	98.1	90.6	95.1	93.0
Horse (citrated)	21	23.9	97.2	92.0	96.8	89.7	95.1	94.2
Beef (fresh)	4	4.5	68.8	95.8	100.0	83.5	89.0	79.2
Sheep ("processed")	10	11.4	92.6	87.0	96.3	91.4	95.9	88.3
Horse with latex	1	1.1	100.0	100.0	100.0	--	100.0	100.0
Total	88	100.0	91.5	94.9	97.6	90.4	95.2	92.5
F Probability			.013	.150	.627	.687	.518	.073

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used).

by the author of the Peterson procedure as necessary for a positive result. It is felt that the high levels of sensitivity, specificity and agreement reported by other investigators (117, 118) is probably more accurate. They reported 98.1% agreement, 97.5% sensitivity and 98.4% specificity for the ox cell test.

Table XXI shows the evaluation of results by type of adsorption used. Most of the laboratories (54.5%) used a procedure which did not involve and adsorption, and most of the laboratories (75%) which used an adsorption procedure used both guinea pig kidney and beef erythrocytes. There were no significant differences among the various adsorption procedures.

Table XXII shows the evaluation of results by end point used. A majority of the laboratories (84.0%) used any visible agglutination as the end point rather than a higher semi-quantitative level. Probably as a result of problems with the ox cell hemolysin tests, the 50% hemolytic end point was significantly lower ($P < .05$) in agreement than the "2+" end point (79.2% vs. 98.7%), and in sensitivity than the agglutination end points (68.8% vs. 92.4%).

Table XXIII shows the evaluation of results by the number of serologic tests performed annually by the laboratory. Although the differences were not significant, there was a gradual increase in agreement as the number of tests performed increased. Specificity was significantly lower ($P < .05$) in the group performing the lower number of tests

TABLE XXI

Evaluation of Results by Type of Absorption Used

Absorption	Number*	Percent of total	Sensitivity	Specificity	Reproducibility Within	Reproducibility Between	Reproducibility Total	Agreement
None	48	54.5	89.4	96.2	98.3	89.7	94.6	92.1
GPK	9	10.2	99.1	89.6	96.0	90.0	95.0	94.3
GPK and BE	30	34.1	92.3	94.3	97.0	91.9	95.8	92.4
HK and BE	1	1.1	100.0	100.0	100.0	--	100.0	100.0
Total	88	100.0	91.5	94.9	97.6	90.4	95.2	92.5
F Probability			.289	.489	.395	.334	.673	.637

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used).

GPK = guinea pig kidney

BE = beef erythrocytes

HK = horse kidney

TABLE XXII

Evaluation of Results of End Point Used

	Number*	Percent of total	Sensitivity	Specificity	Reproducibility Within	Reproducibility Between	Reproducibility Total	Agreement
Any agglutination	68	84.0	92.3	94.3	97.7	91.0	95.4	92.6
1+ agglutination	6	7.4	90.5	96.3	94.5	94.0	94.3	92.8
2+ agglutination	3	3.7	97.3	100.0	97.3	96.0	97.3	98.7
50% hemolysis	4	4.9	68.8	95.8	100.0	83.5	89.0	79.2
Total	81	100.0	91.2	94.8	97.5	90.7	95.1	92.2
F Probability			.016	.587	.312	.499	.305	.077

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used).

TABLE XXIII

Evaluation of Results by Number of Serologic Tests Performed Annually

Number of tests	Number*	Percent of total	Sensitivity	Specificity	Reproducibility Within	Reproducibility Between	Reproducibility Total	Agreement
0 - 250	20	22.7	94.6	88.4	99.2	90.1	95.4	90.0
251 - 1000	29	33.0	89.7	95.9	97.5	94.0	96.3	92.1
1001 - 5000	20	22.7	88.8	98.1	96.2	85.4	92.5	92.6
> 5000	19	21.6	93.8	96.9	97.8	89.6	95.8	95.5
Total	88	100.0	91.5	94.9	97.6	90.4	95.1	92.5
F Probability			.490	.049	.214	.252	.282	.482

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used).

than in any of the other groups (88.4% vs. 96.8%).

Table XXIV shows the evaluation of results by the number of tests for infectious mononucleosis performed annually. Specificity was significantly lower ($P < .05$) in the 0 to 50 test group than it was in the 51 to 500 groups (89.1% vs. 98.4%). The group doing more than 500 test per year achieved a specificity that was not significantly higher than the 0 to 50 test group. Reproducibility was significantly lower ($P < .05$) in the group doing more than 500 infectious mononucleosis tests per year than it was in any of the other groups (89.6% vs. 95.4%). It appears that proficiency in using these tests increases with increased testing to a point after which the proficiency declines to below that of laboratories performing few tests.

Table XXV shows the evaluation of results by the number of technologists in the laboratory. Laboratories with more than 5 technologists achieved significantly lower within shipment reproducibility than the other two groups at the .05 probablility level and significantly lower than the one man laboratories at the .01 probability lever. The one man laboratories obtained 99.3% within shipment reproducibility while the 2 to 5 man and over 5 man laboratories obtained 97.5% and 94.5% respectively. For between shipment reproducibility the laboratories with 2 to 5 technologists were significantly lower ($P < .05$) than the one man laboratories (86.8% vs. 95.9%). The one man laboratories also achieved significantly higher ($P < .05$) total reproducibility than the

TABLE XXIV

Evaluation of Results by Number of Tests for Infectious Mononucleosis Performed Annually

Number of tests	Number*	Percent of total	Sensitivity	Specificity	Reproducibility Within	Reproducibility Between	Reproducibility Total	Agreement
0 - 50	29	33.0	93.1	89.1	97.8	92.1	95.8	90.1
51 - 200	35	39.8	91.7	98.3	98.4	90.9	96.1	94.6
201 - 500	19	21.6	91.7	98.7	95.9	90.6	93.8	94.6
>500	5	5.7	80.0	90.0	98.4	75.3	89.6	84.6
Total	88	100.0	91.5	94.9	97.7	90.4	95.1	92.5
F Probability			.334	.007	.280	.113	.196	.089

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used).

TABLE XXV

Evaluation of Results by Number of Technologists in the Laboratory

Number of technologists	No.*	Percent of total	Sensitivity	Specificity	Reproducibility Within	Reproducibility Between	Reproducibility Total	Agreement
1	22	29.3	92.4	93.2	99.3	95.9	98.0	91.7
2 - 5	36	48.0	88.4	93.7	97.5	86.8	93.3	90.1
> 5	17	22.7	94.8	98.7	94.5	92.1	93.4	96.5
Total	75	100.0	91.0	94.7	97.4	90.6	94.7	92.0
F Probability			.296	.334	.007	.042	.028	.130

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used).

other two groups (98.0% vs. 93.3%).

Table XXVI shows the evaluation of results by the number of technicians in the laboratory. Agreement increased as the number of technicians increased, with the laboratories with more than five technicians achieving significantly better agreement ($P<.05$) than the laboratories with only one technician (95.9% vs. 87.9%). Table XXVII shows the evaluation of results by the total number of laboratory personnel (technicians plus technologists). The laboratories with 2 to 5 people obtained significantly lower sensitivity ($P<.05$) than the one man laboratories (89.6% vs. 96.6%).

Table XXVIII shows the evaluation of results by length of time the specimen was held before testing. Specificity was significantly lower ($P<.05$) for those specimens held longer than one week before testing than it was for those tested within one week (91.5% vs. 97.1%). Reproducibility was significantly lower ($P<.01$) for those specimens held for 4 to 7 days before testing than it was for those tested before or after this time period (94.3% vs. 97.4%).

Table XXIX shows the evaluation of results by specimen holding temperature. Sensitivity and agreement were significantly higher ($P<.05$) for specimens which were held at refrigerator temperatures than for those which were held at room temperature (89.4% vs. 82.4% and 94.9% vs. 90.7% respectively). Reproducibility was significantly better ($P<.05$) for the specimens which were held at room temperature (98.2% vs. 95.5%).

TABLE XXVI

Evaluation of Results by Number of Technicians in the Laboratory

Number of technicians	Number*	Percent of total	Sensitivity	Specificity	Reproducibility Within	Reproducibility Between	Reproducibility Total	Agreement
0	27	30.7	93.2	91.9	98.4	90.6	95.3	91.8
1	16	18.2	87.5	92.7	96.6	88.6	93.8	87.9
2 - 5	29	33.0	90.3	97.8	97.2	91.4	94.8	93.8
>5	16	18.2	94.8	96.9	98.2	89.4	96.9	95.9
Total	88	100.0	91.5	94.9	97.6	90.4	95.1	92.5
F probability			.474	.250	.579	.384	.641	.171

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used).

TABLE XXVII

Evaluation of Results by Number of Personnel (Technicians plus Technologists) in Laboratory

Number of personnel	Number*	Percent of total	Sensitivity	Specificity	Reproducibility Within	Reproducibility Between	Reproducibility Total	Agreement
1	10	11.4	100.0	93.4	99.6	96.4	98.2	96.6
2 - 5	40	45.4	89.2	92.6	97.7	88.8	94.6	89.6
>5	38	43.2	91.7	97.7	97.1	90.2	94.9	94.4
Total	88	100.0	91.5	94.9	97.6	90.4	95.1	92.5
F Probability			.112	.182	.314	.289	.234	.062

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used)

TABLE XXVIII

Evaluation of Results by Length of Time Specimens Held before Testing

Time (Days)	Number*	Percent of total	Sensitivity	Specificity	Reproducibility Within	Between	Total	Agreement
0 - 3	134	62.9	86.3	96.5	98.7	---	98.7	92.6
4 - 7	35	16.4	85.0	99.3	94.3	---	94.3	95.2
> 7	44	20.7	85.8	91.5	93.6	---	93.6	91.3
Total	213	100	86.0	95.9	96.9	---	96.9	92.8
F probability			.335	.043	.001	---	.001	.460

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used).

TABLE XXIX

Evaluation of Results by Specimen Holding Temperature

Temp. (°C)	Number*	Percent of total	Sensitivity	Specificity	Reproducibility Within	Reproducibility Between	Reproducibility Total	Agreement
0 - 15	108	53.7	89.4	96.1	95.5	---	95.5	94.9
16 - 30	93	46.3	82.4	95.2	98.2	---	98.2	90.7
Total	201	100	86.2	95.6	96.8	---	96.8	93.0
F probability			.035	.652	.046	---	.046	.029

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used).

Table XXX shows the evaluation of results by specimen holding time times specimen holding temperature, i.e., the product of the effects of time and temperature. Time times temperature values (TxT) were obtained by the formula:

$$TxT = \text{time} \times 2^{\frac{\text{temperature}}{10}}$$

Where times are in days and temperatures are in degrees C. This relationship was used to reflect the common characteristic of many biological phenomena of approximately doubling the effect due to time with each 10 C increase in temperature. For example, any specimen tested on the day it arrived would have a time of 0 days and a TxT value of 0 regardless of the temperature at which it was held. A specimen which was tested on the day after it was received would have a time of 1 day and the TxT values would be 1 if it had been held at 0 C, 2 if it had been held at 10 C, 4 if it had been held at 20 C, etc.. Thus, specimens held for 1 day at 20 C would have the same TxT value as specimens held for 2 days at 10 C, i.e., 4.

Sensitivity, specificity, reproducibility and agreement were all from 2 to 6 percentage points lower in the specimens with the lower TxT values than the group which was tested sooner and held at lower temperature, but none of the differences were significant.

Table XXXI shows the cost and time involved in the various types of tests as reported by the participants. There was no significant difference between the costs and

TABLE XXX

Evaluation of Results by Specimen Holding Time Times Specimen Holding Temperature

T X T	Number*	Percent of total	Sensitivity	Specificity	Reproducibility Within	Between	Total	Agreement
0 - 7	133	49.4	88.1	97.0	97.8	---	97.8	93.9
> 7	80	50.6	82.5	94.1	95.4	---	95.4	90.9
Total	213	100	86.0	95.9	96.9	---	96.9	92.8
F probability			.088	.150	.061	---	.061	.121

* indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used).

TABLE XXXI

Reported Costs by Type of Test

Test	Time (min)	Time cost ($)	Material cost ($)	Total cost ($)	No. of reports
Bacto-Hetrol (Difco)	12.5 ± 2.9	1.08 ± .38	.60 ± .49	1.68 ± .36	5
Davidsohn Differential	35.0 ± 8.7	2.12 ± .12	.46 ± .05	2.58 ± .11	3
Monotest (Wampole)	7.0 ± 5.8	0.60 ± .49	.47 ± .24	1.07 ± .53	24
Monospot (Ortho)	6.7 ± 2.5	0.43 ± .17	1.36 ± .81	1.70 ± .60	9
Monosticon (Organon)	6.6 ± 3.1	0.46 ± .20	1.48 ± 1.11	1.94 ± .82	10
Ox-Cell Hemolysin	25.0 ± 17.3	1.75 ± .43	.63 ± .29	2.38 ± .72	3
Diagluto (Beckman)	7.8 ± 4.7	0.49 ± .31	.70 ± .56	1.38 ± .45	5
I.M. Kit (MicroRshCorp)	5.2 ± 2.1	0.58 ± .39	.61 ± .28	1.19 ± .58	9
Dri-Dot (Organon)	6.4 ± 3.4	0.61 ± .32	.99 ± .40	1.60 ± .40	7
Mono-Diff (Wampole)	5.0 ± 0	0.25 ± 0	2.00 ± 0	2.25 ± 0	1
Monophile (Bio-Diag-Sys)	2.0 ± 0	0.14 ± 0	1.00 ± 0	1.14 ± 0	1
Rythrotex (BMC)	6.0 ± 0	0.42 ± 0	.37 ± 0	.79 ± 0	1
$\bar{x}$ ± SD	10.4 ± 9.7	0.74 ± .61	.89 ± .50	1.61 ± .55	78
Tube test total	30.0 ± 7.1	1.94 ± .26	.54 ± .12	2.48 ± .14	2
Slide test total	6.5 ± 2.6	.51 ± .25	.96 ± .52	1.46 ± .44	10
Total	10.4 ± 9.7	0.74 ± .61	.89 ± .50	1.61 ± .55	12

times reported for the Davidsohn differential and the ox cell hemolysin tests. There were no significant differences among the slide tests for either costs or time required. The tube tests cost less for material but more for technologist time than the slide tests. The overall costs were about $1.00 per test higher for the tube tests than for the slide tests.

In calculating the cost of technologist's time on those reports in which the time was reported but no cost for technologist's time was reported, it was assumed that the average salary for a medical technologist was $675 per month or $0.07 per minute. Cost of technologist's time was calculated accordingly.

Table XXXII shows the results of subjective evaluation of tests for infectious mononucleosis as reported by the laboratories using the tests. Some of the evaluations are inaccurate and some list the same characteristic as both an advantage and a disadvantage. Speed and ease of performance were the most frequently mentioned advantages and cost and lack of titer capability were the most frequently mentioned disadvantages.

Table XXXIII shows a comparison of the participants reported titers with the established target values. The ox cell hemolysin titers reported by the participants seem to be a little low but the target values were well within the two standard deviation range in each case. The ox cell hemolysin titers reported by CDC correlated much more closely

TABLE XXXII
Tabulation of Subjective Evaluation of Tests for Infectious Mononucleosis

	Bacto-Hetrol (Difco)		Davidsohn Differential		Monotest (Wampole)		Monospot (Ortho)		Monosticon (Organon)		Ox-Cell Hemolysin		Diagluto (Beckman)		I.M. Kit (MicroRshCorp)		Dri-Dot (Organon)		Mono-Diff (Wampole)		Monophile (Bio-Diag-Sys)		Rythrotex (BMC)		Total	
	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A	D	A
Standard			1																						-	1
Specificity			1		1	2			1		1								1						1	6
Sensitivity					2						2														-	4
Speed			1		12		3		3		1				6				1						2	26
Ease of performance					10								1		4		1								1	16
Ease of reading					2	2	1						1		1	2	1						1		3	8
Cost					1	2			1	1			1		4		1[d]		1						4	7
Titration					3	3					1														4	4
Shelf-life					2				1				2		1		1								1	7
Control provided					1[a]	1									2[e,g]										1	3
Accuracy					1	3	2		3				1		3										1	12
Convenience					1		1[f]		1								1								2	3
Screen only									2																2	-
Screen and confirm							1																		-	1
Differential					2				1				2												2	3
Correlation clin. & hemat.					1[b]		1[c]		1																1	2
No glassware required															1										-	1
Test evaluated					1				1																2	-
Uses formalinized not fresh cells																									1	-
Total	0	0	1	2	12	41	2	7	4	12	0	5	1	8	4	23	1	4	1	2	0	0	0	1		

a = control too positive
b = positive prior to clinical symptoms
c = heterophile antibodies don't show u until after atypical lymphs appear in blood
d = expense of dilutions
e = human positive control
f = only one specimen at a time
g = positive and negative controls
h = cannot be done on plasma or from

TABLE XXXIII

Comparison of Participants' Results with Target Values

Pool #	Ox-Cell Hemolysin		Davidsohn Differential Unabsorbed		GPK Absorbed		BE Absorbed	
	Target	Test	Target	Test	Target	Test	Target	Test
1	80	2.00 x/÷ 7.64	56	33.3 x/÷ 1.41	≥7 =	12.7 x/÷ 4.12	<7	3.00 x/÷ 3.32
2	160	30.8 x/÷ 2.28	112	224	≥14 =	83.9 x/÷ 1.58	<7	1.18 x/÷ 2.93
3	320	71.3 x/÷ 2.53*	224	266.4 x/÷ 1.41	≥28 =	79.2 x/÷ 1.49	<7	<7
4	<10	<10	<7	<7	<7	<7	<7	<7
5	<10	<10	<7	14	<7	<7	<7	<7
6	<10	2.89 x/÷ 4.91	56	224	56	133.2 x/÷ 1.41	56	224

* includes four titers of 240 by the Peterson method which were converted to 80 which is the CDC method equivalent.

with the target values than did those from the other two laboratories. Since the CDC results were from a laboratory using a well established procedure and employing technologists familiar with the test and the other two laboratories were using either new procedures or new personnel, the CDC results were considered the most reliable.

All of the Davidsohn differential target titers were well within the two standard deviation range reported by the participants except for those with no deviations and the Davidsohn differential guinea pig kidney absorbed titers on pool #6 which were based on only 4 titrations.

The results of titrations were reported as mean x/÷ one standard deviation. When working with arithmetic data means and standard deviations are commonly expressed in the form $\bar{x} \pm SD$. Since the distribution of values about a logarithmic or geometric mean is not arithmetically symmetrical, this type of notation is not applicable and other more cumbersome methods are commonly employed. Two methods which are frequently resorted to are to express the standard deviation in terms of the applicable ranges or to simply leave the mean and standard deviation in the logarithmic form and not express them as antilogs at all. Both of these alternatives are awkward and inconvenient to use especially for those with little daily exposure to such manuvers.

The simple notation used in this paper for indicating geometric means and standard deviations is in the form $\bar{x}_G$ x/÷ SD_G which is analogous to the arithmetic $\bar{x} \pm SD$.

In other words, the one standard deviation range includes all values between $\bar{x}_G \div SD_G$ and $\bar{x}_G \times SD_G$. To obtain ranges other than one standard deviation the SD_G is raised to the power of the number of standard deviations desired. For example, the two standard deviation range would include all values between $\bar{x}_G \div (SD_G)^2$ and $\bar{x}_G \times (SD_G)^2$; the three standard deviation range would include all those values between $\bar{x}_G \div (SD_G)^3$ and $\bar{x}_G \times (SD_G)^3$.

Use of this simple notation makes the figures much easier to handle, more readily understood and easier to compare or use for further calculations.

One laboratory using the CDC ox cell hemolysin procedure observed that some lots of dehydrated complement exhibited a small amount of hemolytic activity in the absence of serum. Consequently, they felt that it was desirable to include a complement control in the experimental procedure. They were apparently not following the CDC procedure closely since it specifies the inclusion of complement-control. An alternative would be to test each lot of complement before it is used.

DISCUSSION

An important area of serologic proficiency testing which this study begins to investigate is the relative value of the different procedures used in laboratories. For a given serologic examination the test procedures vary extensively from laboratory to laboratory. Nothing which resembles a standard reference procedure (except in syphilis serology) is in existance and there is little if any information concerning the relative specificity, sensitivity and reproducibility of the various serologic techniques used.

This study shows that proficiency testing can be so designed that the results can be used as an evaluation survey to allow collection and correlation of data concerning the relative efficiency of the tests used. The main problem to be overcome in such an endeavor is to obtain enough data on single pools of sera to allow confident statements to be made. Large pools were made and stored in small aliquots to be shipped and tested at different times and by different shipments. The resultant data gives a good idea of the sensitivity, specificity and reproducibility of some of the tests as they are employed in the laboratories and the effect of some of the variables on the performance of the tests.

Poor response was received from the recruitions letters

which were mailed to potential participants. Only 20 of the 53 laboratories which ultimately participated in the study responded to the letters. Most of the participants were recruited by means of telephone calls just prior to the first shipping date. Many of the laboratories indicated that they desired to participate but had just failed to fill out and return the forms. In projects of this type in the future it would probably be advantageous to send only a self addressed post card to determine which laboratories wanted to participate. This card should have spaces for indication of positive and negative responses and should be returned in either case. Cards not returned after a specified time could then be followed up with much less difficulty. After the list of participant laboratories had been established, then the background and test procedure questionnaires could be mailed.

Even though considerable effort was expended to make sure that the questionnaire was clear and unambiguous and the forms were designed so the data could be keypunched directly, these efforts met with little success. Many forms were returned incomplete. In some cases all the possible choices were circled when the instructions clearly indicated "circle one". Often the data entered did not fit the spaces that were provided. These results indicate that it would be worth while to submit the questionnaires to extensive evaluation prior to use. The forms should be completed by the same type of people who will be using it during the

evaluation with any problems or questions returned to the person designing the form.

Since the average values for each of the parameters measured was over 90% in most cases and since many laboratories obtained values of 100%, it was difficult to obtain results which were significantly different. To obtain significant differences on tests with performance levels as high as these would probably require the use of a nationwide proficiency testing program.

One of the limitations of this study was that very few of the laboratories reported titers and many did not indicate weak positive reactions and as a consequence, the evaluation was not as sensitive as it might have been. Smaller differences in test performance could probably have been determined if more titration procedures were involved.

A considerable amount of time was spent on the data processing phase of this project. A computer program which had only recently been added to the university's computer program library was used to perform the analyses of variance. As a result, our use of this program served the additional purpose of "debugging" the program. At the termination of the study the "debugging" was still not completed.

A great deal of time was also spent in checking the original reports for internal consistency, in checking cards images of these reports and in correcting and verifying reporting and keypunching errors. These problems plus a number of changes in the way data were handled resulted in

a large number of computer output products from which extractions were made.

This experience with automated data processing emphasized the importance of a close liaison between the experimenter and the programmer. It is helpful if each is familiar with the work of the other.

SUMMARY

Monotest (Wampole) was by far the most frequently used test in this evaluation (27/88 or 30.7%) with Monospot (Ortho) (13/88 or 14.8%), Monosticon (Organon) (10/88 or 11.4%), I.M. Kit (Micro. Res. Corp.) (10/88 or 11.4%), Diagluto I.M. (Beckman) (8/88 or 9.1%) and Monosticon Dri-Dot (Organon) (7/88 or 8.0%) following.

Of the tests used by three or more laboratories the most sensitive was Monosticon Dri-Dot (Organon) (100%) and the least sensitive was the ox cell hemolysin (68.8%). The Davidsohn differential and Diagluto I.M. (Beckman) both achieved 100% specificity; Monosticon (Organon) and Monosticon Dri-Dot (Organon) had the lowest specificity (87.0% and 86.6% respectively). There was an inversely proportional relationship between sensitivity and specificity ($P < .02$), i.e., as sensitivity increased specificity decreased.

Within shipment reproducibility was consistently about 5 percentage points higher than the between shipment reproducibility ($P < .001$).

The Davidsohn differential obtained the best agreement with target values but it was not significantly better than the slide tests. None of the differences in tests were significant.

Although there were a number of changes in test use, frequently multiple changes, eighty percent of the laboratories did not change tests during the evaluation.

Performance improved during the evaluation as revealed by the fact that agreement was 5 percentage points higher for the second half than it was for the first half ($P < .05$). Presumably this improvement was attributable to increased proficiency which resulted from the educational and corrective influence of proficiency testing.

Private hospitals achieved significantly lower sensitivity ($P < .01$) and agreement ($P < .05$) than other types of laboratory. Sensitivity was 69.0% versus 93.3% and agreement was 82.2% versus 92.8%. Thus, private hospitals were more likely to miss the serologic diagnosis in a case of infectious mononucleosis.

Technologists with a baccalaureate degree achieved a little better agreement (92.8%) than did either the high school graduates (90.0%) or the technologists with masters degrees (89.8%) but the differences were not statistically significant. The one "technologist" with a doctorate did very well (100% agreement, specificity and sensitivity) but his performance was not significantly better than any other group.

The two I.S.C.L.T. registered technologists were about 10 percentage points lower in agreement (79.5% vs. 92.8%) than the other groups (A.S.C.P., A.M.T. and non-registered technologists). Sensitivity of tests run was also lower

for this group (66.5% vs. 92.1%).

Experience of the technologist made little difference in the results but in general the group with 3 to 10 years of experience performed the best, followed by the group with 0 to 2 years, and the group with more than 10 years experience did the poorest. Experience with the particular test being used had about the same results. Technologists with 3 or more years of experience with the particular test had significantly lower ($P < .05$) sensitivity (85.2% vs. 93.8%) than the other group. The hypothesis formulated from this observation is that experience enhances performance up to a point after which performance deteriorates to a level lower than shown by those with no experience. This suggests that evaluations of technologist's performance needs to be an ongoing effort and should not be discontinued after minimum qualifications have been achieved. Also, it seems likely that continued interest in the tests being performed is an important factor in maintaining proficiency.

Most laboratories (54.5%) used a procedure which did not involve any absorption, and most of the laboratories (75%) which used an absorption procedure used both guinea pig kidney and beef erythrocytes. There were no significant differences among the various absorption procedures.

There was no significant differences among the different agglutination end points and the significant differences in the 50% hemolytic end point was probably not due to the test itself.

Both the length of time the specimens were held before testing and the temperature at which they were held had an effect on the reliability of the test. Specificity, reproducibility, and agreement were all lower for specimens held over one week before testing than they were for those tested within one week. Sensitivity, specificity and agreement were all lower for specimens held at room temperature than they were for specimens held at refrigerator temperatures, but reproducibility was higher when specimens were held at room temperature. When the combined effects of time and temperature were evaluated it was observed that all of the parameters were 2 to 6 percentage points lower for the specimens which were held the longest times and at the highest temperatures.

We were unable to evaluate the effect of controls since 96% of the laboratories used at least a positive control.

Slightly more than half of the laboratories (52.4%) used the same test as both screen and confirmation and many laboratories (41.5%) used the tests only as screens. There were no significant differences in tests by use, but laboratories that used their test as a confirmatory test had better agreement (96.0% vs. 92.6%) and higher specificity (100% vs. 94.9%) than laboratories that used the test for screening only or for both.

Most laboratories (79.8%) used tests in which the technologists had "good" confidence and none of the laboratories used tests in which the technologists had

"poor" confidence. Although none of the differences were significant, the tests in which the technologists had only "fair" confidence performed slightly better in general than did the tests in which the technologist expressed "good" confidence.

In general, technologists specializing in hematology or blood banking obtained the best results, with bacteriologists and serologists next. Chemists and general medical technologists obtain the poorest results. It seems that familiarity with the theory of serologic agglutination tests and some of the possible sources of error in these tests could be important here.

Beef cells yielded significantly lower ($P < .05$) agreement (79.2% vs. 93.1%) than fresh sheep cells or the three groups of horse cells. The beef cells were also less sensitive ($P < .01$) than the other cells (68.8% vs. 92.6%). There were no significant differences in type of absorption or end point.

As the number of serologic tests performed annually in the laboratory increased the performance increased although some of the differences were not significant. Similar results were observed with the number of tests for infectious mononucleosis performed in the laboratory, but there was a point after which performance declined. This could be explained by the proposal that "practice makes perfect" but if the procedure becomes monotonous the proficiency is adversely affected.

One man (technologist) laboratories achieved significantly higher ($P<.05$) total reproducibility (98.0% vs. 93.3%) than other laboratories. Agreement increased as the number of technicians increased, with the laboratory with more than five technicians achieving significantly better ($P<.05$) agreement (95.9% vs. 91.7%) than laboratories with only one technician. Higher levels of performance in laboratories with larger numbers of personnel could be the result of specialization.

There were no significant differences between the reported cost and time requirements for the two standard tests, nor among the slide tests. The standard tests required 30.0 $\pm$ 7.1 minutes to perform and cost \$2.48 $\pm$ .14 for cost of materials and technologist's time. The slide tests required 6.5 $\pm$ 2.6 minutes to perform and cost \$1.46 $\pm$ .44 for materials and technologist's time.

Subjective evaluation of the tests showed that speed and ease of performance were the most frequently mentioned advantages and cost and lack of titer were the most frequently mentioned disadvantages.

It seems that a small laboratory could do satisfactory serologic testing for infectious mononucleosis by selecting one of the slide tests that meet their requirements for cost, shelf life, titer capability, etc., and using it with proper controls. For larger laboratories the slide tests would serve well as screens with the standard tests (Davidsohn differential or ox cell hemolysin) used for

titration when requested.

Even though most of the parameters measured and variables evaluated exhibited substantial differences they were usually not significantly different due to the small sample size in some cases and large variation in results in most cases. This points out the need for similar evaluations using larger proficiency testing programs. Programs similar to the one described here should be conducted to evaluate other types of tests so that test selection can be made on the basis of measured effectiveness rather than the methods currently used.

APPENDICES

STATE OF UTAH–DEPARTMENT OF SOCIAL SERVICES

CALVIN L. RAMPTON
Governor

PAUL S. ROSE
Executive Director

DIVISION OF HEALTH
44 MEDICAL DRIVE
SALT LAKE CITY, UTAH 84113
AREA CODE 801

Board of Health
Air Conservation Committee
Health Facilities Council
Medical Examiner Committee
Nursing Home Advisory Council
Water Pollution Committee

STYMAN J. OLSEN, M.D., M.P.H.
Director of Health

BUREAU OF LABORATORIES

December 14, 1973

MEMORANDUM

TO: All Utah Clinical Laboratories, Plus Reference Laboratories

FROM: Laboratory Improvement Program

SUBJECT: Request for Participation in Evaluation of Tests for Infectious Mononucleosis

The Utah State Division of Health is undertaking a project to evaluate the sensitivity, specificity, and reproducibility of the various tests used in the state to detect infectious mononucleosis. In order to accomplish this, we plan to modify the immunology proficiency testing program so that the additional information can be obtained as well as the routine proficiency testing data. These modifications will include requests for laboratory and technologist background information and details of the test procedure.

Laboratories enrolled in the immunology proficiency testing program and reference laboratories will be automatically included in this evaluation survey since it will also serve to fill the proficiency testing requirements for state approval. It is hoped that these laboratories will not find the extra work involved excessive and will be able to complete the required forms.

Other laboratories within the state are requested and encouraged to participate in this program on a one-time basis so that this evaluation will accurately reflect the reliability of infectious mononucleosis tests used within the state. The evaluation will consist of four shipments of six specimens, each to be tested for infectious mononucleosis by whatever method is routinely used in the laboratory. During the evaluation, laboratories will be coded so that only the Division of Health and the individual laboratories will be able to identify their results. At the conclusion of the study, a project report will be sent to each participant summarizing our findings and acknowledging the participation of cooperating laboratories.

Laboratories willing to participate in this project should fill out the enclosed Background Questionnaire and Test Procedures forms and return them to the Division of Health. If more than one test is used in the laboratory, complete one Test Procedure form for each test. The only other information required will be the Test Reports to be completed upon receipt of each shipment of specimens. If tests used or technologist performing the tests are changed during the

course of this study it will be necessary to update the Background Questionnaire or Test Procedure form applicable.

We hope that you will be able to cooperate in this study since it is designed to provide critically needed information which will permit decisions concerning test selection to be based on reliable evaluation results.

Please complete and return the enclosed forms as soon as possible. If you have any questions concerning this program please contact me.

Sincerely,

Roger N. Taylor, Microbiologist
Laboratory Improvement Program

Appendix B

BACKGROUND QUESTIONNAIRE

Date_____

Lab. No. ___ Lab. name _____
(Col. 1-2)

Test employed
(circle one) (Col. 3)
1. Paul-Bunnel Presumptive
2. Davidsohn Differential
3. Monotest (Wampole)
4. Monospot (Ortho)
5. Monosticon (Organon)
6. Ox-cell Hemolysin
7. Diagluto I.M. (Beckman)
8. I.M. Kit (Micro. Sc.)
9. Other (specify) _____

Technologist performing test _____ (name)
(Col. 4)

Formal education
(circle one) (Col.5)
1. High school
2. B.S.
3. M.S.
4. Ph.D.

Registration
(circle one) (Col. 6)
1. ASCP
2. AMT
3. Other (specify _____
4. None

Years of experience __ __ (Col. 7-8)

Area of major interest
(circle one) (Col.9)
1. Chemistry
2. Bacteriology
3. Serology
4. Hematology
5. Blood banking
6. Other (specify) _____

Type of laboratory
(circle one)
(Col. 10)
1. Private hospital
2. Government hospital (city, county, etc.)
3. Clinic
4. Independent
5. Other (specify _____

Number of technologists in laboratory __ __
(Col. 11-12)

Number of technicians in laboratory __ __
(Col. 13-14)

Number of serologic tests performed in laboratory per year __ __ __ __
(Col. 15-18)

Number of tests for I.M. performed in laboratory per year __ __ __ __
(Col. 19-22)

Col. 80 = 2

Appendix C

TEST PROCEDURES
(Answer all applicable questions)

Date _____

Lab. No. __ __ Lab. Name _____
(Col. 1-2)

Test employed:
(circle one) (Col. 3)
1. Paul-Bunnell Presumptive
2. Davidsohn Differential
3. Monotest (Wampole)
4. Monospot (Ortho)
5. Monosticon (Organon)
6. Ox-cell Hemolysin
7. Diagluto I.M. (Beckman)
8. I.M. Kit (Micro. Sc.)
9. Other (specify) _____

Type of indicator cells
(circle one) (Col. 4)
1. Sheep (fresh)
2. Horse (formalinized)
3. Horse (citrated)
4. Beef (fresh)
5. Other (specify) _____

Absorption
(circle one) (Col. 5)
1. None
2. Guinea pig kidney
3. Beef Erythrocyte
4. G.P.K. and B.E.
5. Other (specify) _____

Cell suspension __ __ % (Col. 6-7)
C' dilution 1: __ __ (Col. 8-9)

Diluent
(circle one) (Col. 10)
1. Saline
2. Veronal Buffer
3. Other (specify) _____

Titration scheme
(Col. 11-12) Initial dilution 1: __ __
(Col. 13-14) Ratio (2 fold, 10 fold, etc.)
__ __ fold

Incubation time __ __ min. (Col. 15-16)
Incubation temp. __ __ ° C (Col. 17-18)
Refrigerator time __ __ hrs. (Col. 19-20)
Refrigerator temp. __ __ ° C (Col. 21-22)
Centrifuge time __ __ min. (Col. 23-24)
Centrifuge speed __ __ g's x 100 (Col. 25-26)
Centrifuge # of times __ __ (Col. 27-28)

End point reaction
(circle one) (Col. 29)
1. Any agglutination
2. 1+
3. 2+
4. 3+
5. 4+
6. 50% hemolysis
7. 100% hemolysis
8. Other (specify) _____

Dilutions reported
(circle one) (Col. 30)
1. Initial (before cells added)
2. Final (after cells added)

Significant titers
Unabsorbed __ __ __ (Col. 31-33)
G.P. Abs. __ __ __ (Col. 34-36)
B.E. Abs. __ __ __ (Col. 37-39)

Use of test
(Circle one) (Col. 40)
1. Screen
2. Confirm
3. Both

Number of years of experience with this test __ __ (Col. 41-42)

Technologists confidence in this test
(circle one) (Col. 43)
1. Good
2. Fair
3. Poor

Estimated cost per test for materials
$ __ __. __ __ (Col. 44-47)

Estimated cost per test for tech. time
$ __ __. __ __ (Col. 48-51)

Average tech. time required for one test
__ __ min. (Col. 52-53)

Advantages of this test (list)

Disadvantages of this test (list)

Col. 80 = 1

Appendix D

INFORMATION AND INSTRUCTION SHEET FOR
UTAH STATE DIVISION OF HEALTH
PROFICIENCY TESTING SAMPLES AND
EVALUATION SURVEY

INFECTIOUS MONONUCLEOSIS

1. This series of specimens is to be tested for infectious mononucleosis.

2. Please test these specimens using method currently employed by your laboratory.

3. Have reports signed by both the person performing the examination and by the supervisor.

4. Forward reports to this office by May 20,1974.

5. Please indicate the following on the enclosed report form(s).

   a. Positive or negative reactions for each specimen submitted and the titer for each positive specimen if a titration procedure is done in your laboratory.

   b. Please complete and return the appropriate Test Procedures Form and/or Background Questionnaire if they have not previously been submitted to the Utah State Division of Health for the procedure and/or technologist performing this set of tests. If more than one test is used, a separate Test Procedure Form should be completed for each. If more than one technologist performs tests in this program, a Background Questionnaire should be completed for each. If we already have this information, these forms need not be filled out.

6. Specimens consist of whole serum and needs no reconstitution.

Appendix E

TEST REPORT

Date _____

Laboratory No. __ __ (Col. 1-2) Laboratory name _____

Test employed: (circle one) (Col. 3)

1. Paul-Bunnell Presumptive
2. Davidsohn Differential
3. Monotest (Wampole)
4. Monospot (Ortho)
5. Monosticon (Organon)
6. Ox-cell Hemolysin
7. Diagluto I.M. (Beckman)
8. I.M. Kit (Micro. Sc.)
9. Other (specify) _____

Technonogist name _____ (Col. 4)

Quarter __ (Col. 5)

Date specimen received: __ __ __ __ (Col. 6-9)
day month

Date specimen tested: __ __ __ __ (Col. 10-13)
day month

Controls used: (Col. 14)
1. Positive
2. Negative
3. Positive and negative

Holding temperature __ __ ° C. (Col. 15-16)

RESULTS

Do not use this space. Office use only	Specimen #	TITERS			INTERPRETATION
		Unabs.	G.P. Abs.	B.E. Abs.	(+) Pos. or (-) Neg.
(Col.17)		1: _ _ _ _ (Col. 18-21)	1: _ _ _ _ (Col.22-25)	1: _ _ _ _ (Col. 26-30)	(Col. 31)
(Col. 32		1: _ _ _ _ (Col. 33-36)	1: _ _ _ _ (Col.37-40)	1: _ _ _ _ (Col. 41-44)	(Col.45)
(Col. 46		1: _ _ _ _ (Col. 47-50)	1: _ _ _ _ (Col. 51-54)	1: _ _ _ _ (Col. 55-58)	(Col. 59)
(Col. 60)		1: _ _ _ _ (Col. 61-64)	1: _ _ _ _ (Col. 65-68)	1: _ _ _ _ (Col. 69-72)	(Col. 73)
(Col. 74)		1: _ _ _ _ (Col. 75-78)	1: _ _ _ _ *(Col. 5-8)	1: _ _ _ _ (Col. 9-12)	(Col. 13)
(Col. 14)		1: _ _ _ _ (Col. 15-18)	1: _ _ _ _ (Col. 19-22)	1: _ _ _ _ (Col. 23-26)	(Col. 27)

* Dup. Col. 1-4

Col. 80 = 3

APPENDIX F

List of Participant Laboratories

I. W. Allen Memorial Hospital
719 West 4th North
Moab, Utah 84532

American Fork Hospital
350 East 300 North
American Fork, Utah 84003

Beaver Valley Hospital
85 North 400 East
Beaver, Utah 84713

Brigham Medical Clinic, Inc.
1400 North 1st East
Brigham City, Utah 84302

Budge Clinic
225 East 4th North
Logan, Utah 84321

B.Y.U. Student Health
Brigham Young University
Provo, Utah 84601

Carbon County Hospital
Washington Park
Price, Utah 84501

Cooley Memorial Hospital
40 North 1st East
Brigham City, Utah 84302

Cottonwood Hospital
5770 South 3rd East
Murray, Utah 84121

Dixie Memorial Hospital
551 South 300 East
St. George, Utah 84770

Duchesne County Hospital
26 West 2nd North
P.O. Box 698
Roosevelt, Utah 84066

Fillmore LDS Hospital
25 South 100 West
Fillmore, Utah 84631

General Health Systems
4500 South 1900 West
Roy, Utah 84067

Gunnison Valley Hospital
P.O. Box 354
Gunnison, Utah 84634

Holy Cross Hospital
1045 East 1st South
Salt Lake City, Utah 84102

Intermountain Clinic
699 East South Temple
Salt Lake City, Utah 84102

Intermountain Laboratories, Inc.
870 East 7200 South
Midvale, Utah 84047

Juab County Hospital
549 North 4th East
Nephi, Utah 84648

Logan LDS Hospital
218 North 3rd East
Logan, Utah 84321

McKay-Dee Memorial Hospital
3939 Harrison Avenue
Ogden, Utah 84403

Medical Center Laboratory
508 East South Temple #120
Salt Lake City, Utah 84102

Microbiological Research Corp.
481 South 400 East
Bountiful, Utah 84010

Neighborhood Health Center
127 East 21st South
Salt Lake City, Utah 84115

Ogden Clinic
2955 Harrison Avenue
Ogden, Utah 84403

Panguitch LDS Hospital
145 East Center
Panguitch, Utah 84759

Pathology Associates Laboratory
1965 East 5600 South
Salt Lake City, Utah 84121

Primary Childrens Hospital
320 12th Avenue
Salt Lake City, Utah 84103

St. Mark's Hospital
1200 East 3900 South
Salt Lake City, Utah 84117

Salt Lake LDS Hospital
325 8th Avenue
Salt Lake City, Utah 84103

Sevier Valley LDS Hospital
201 East 500 North
Richfield, Utah 84701

Tanner Memorial Clinic
312 Gentile
Layton, Utah 84041

Uintah County Hospital
175 North 1st West
Vernal, Utah 84078

U.S.A.F. Hospital
Hill Air Force Base
Clearfield, Utah 84406

Utah State Training School
P.O. Box 8
American Fork, Utah 84003

Valley View Medical Center
595 South 75 East
Cedar City, Utah 84720

Veterans Administration Hosp.
500 Foothill Drive
Salt Lake City, Utah 84113

West Millard Hospital
275 West 100 South
Delta, Utah 84624

Para Diagnostics Laboratory
2180 East 4500 South
Salt Lake City, Utah

Physicians Clinical Laboratory
2036 South 13th East
Salt Lake City, Utah 84105

St. Benedict's Hospital
3000 Polk Avenue
Ogden, Utah 84403

Salt Lake Clinic
333 South 9th East
Salt Lake City, Utah 84102

San Juan Hospital
384 West 3rd North
Monticello, Utah 84536

South Davis Community Hospital
401 South 400 East
Bountiful, Utah 84010

Tooele Valley Hospital
211 South 1st East
Tooele, Utah 84074

University Medical Center
50 North Medical Drive
Salt Lake City, Utah 84112

U.S. Army Hospital
Dugway Proving Ground
Dugway, Utah 84022

Utah Valley LDS Hospital
1034 North 5th West
Provo, Utah 84601

Valley West Hospital
4160 West 3400 South
Granger, Utah 84102

Wasatch Laboratories
211 Medical Arts Building
Salt Lake City, Utah 84110

Referee Laboratories

Center for Disease Control
Microbiology and Serology Unit
Atlanta, Georgia 30333

Department of Health & Environmental Sciences
Laboratory Division
Helena, Montana 59601

Utah State Division of Health
Serology Section
Salt Lake City, Utah 84113

Appendix G

STATE OF UTAH—DEPARTMENT OF SOCIAL SERVICES

CALVIN L. RAMPTON
Governor

PAUL S. ROSE
Executive Director

DIVISION OF HEALTH
44 MEDICAL DRIVE
SALT LAKE CITY, UTAH 84113
AREA CODE 801
328-6131

Board of Health
Air Conservation Committee
Health Facilities Council
Medical Examiner Committee
Nursing Home Advisory Council
Water Pollution Committee

BUREAU OF LABORATORIES

LYMAN J. OLSEN, M.D., M.P.H.
Director of Health

January 4, 1974

MEMORANDUM

TO: Participants, Immunology Proficiency Testing Program and Infectious Mononucleosis Test Evaluation Program

FROM: Laboratory Improvement Program, Bureau of Laboratories

SUBJECT: Shipment No. 1, 1974, Infectious Mononucleosis Testing

Under separate cover we are sending six (6) specimens for examination as part of the Utah State Proficiency Testing Program. The specimens should be examined and the results reported to this office no later than January 21, 1974.

An instruction sheet will be enclosed with the specimens. We will also send two report forms in order that you may retain a copy for your files.

A report of the findings of the reference laboratories and the participant laboratories will be forwarded upon receipt of the results.

Sincerely,

John L. Clayton

John L. Clayton, Microbiologist

JLC/eh

Appendix H

STATE OF UTAH-DEPARTMENT OF SOCIAL SERVICES

CALVIN L. RAMPTON
Governor

PAUL S. ROSE
Executive Director

DIVISION OF HEALTH
44 MEDICAL DRIVE
SALT LAKE CITY, UTAH 84113
AREA CODE 801
328-6131

Board of Health
Air Conservation Committee
Health Facilities Council
Medical Examiner Committee
Nursing Home Advisory Council
Water Pollution Committee

LYMAN J. OLSEN, M.D., M.P.H.
Director of Health

BUREAU OF LABORATORIES

February 6, 1974

MEMORANDUM

TO: Participants, Immunology Proficiency Testing Program and Infectious Mononucleosis Test Evaluation Program

FROM: Laboratory Improvement Program, Bureau of Laboratories

SUBJECT: I. M. Test Results - Shipment No. 1, 1974

On 7 January 1974 six infectious mononucleosis test evaluation and proficiency testing specimens were shipped to participating laboratories. Table I shows the results obtained by each laboratory and their score.

The target values for these samples were as follows:

Specimen No.	Slide Tests	Ox-Cell Hemolysin (CDC Method)	Davidsohn Differential		
			Unabs.	G.P.K. Abs.	B.E. Abs.
SB-1-74 and SB-2-74	Neg	<10	< 7	< 7	< 7
SB-3-74 and SB-4-74	Pos	80	56	≥ 7	< 7
SB-5-74 and SB-6-74	Pos	320	224	≥ 28	< 7

For state approval in immunology, laboratories are required to obtain at least 75% agreement with referee laboratories. In this set of specimens the referee laboratories (Lab. Nos. 70, 71, and 72) received an average grade of 77.7%. Therefore, the minimum acceptable grade for state approval on this shipment is 75% of 77.7%, or 58.3%. All laboratories received acceptable scores due to the poor performance of the referee laboratories. Of the 50 test reports returned, 35 received grades of 100%; 4 received grades of 83% and 12 received grades of 67%. The average (mean) grade was 90.9%. The average score with the standard tests (Davidsohn Differential and Ox-Cell Hemolysin) was 80.0% while the average score for the slide tests was 92.1%.

All of the erroneous results were reported on specimens SB-3-74 and SB-4-74 which were made from the same pool and were intended to be borderline positives.

Of the test reports on these two specimens 51.0% were positive; 21.6% were weakly positive and 27.4% were negative.

Laboratories are encouraged to review their procedures to verify that they are performed in accordance with the author's or manufacturer's directions and that proper controls are included.

Your participation in this program is appreciated. If there are any questions concerning the program or your results please contact us.

Sincerely,

John L. Clayton, Microbiologist

Roger N. Taylor, Microbiologist

JLC/RNT/eh

TABLE I

Immunology Proficiency Testing and Infectious Mononucleosis Test Evaluation Results

Sample Nos. / Lab. Nos.	SB-1-74	SB-2-74	SB-3-74	SB-4-74	SB-5-74	SB-6-74	Score
1	Neg	Neg	Pos	Pos	Pos	Pos.	100
2	Neg	Neg	W. Pos	W. Pos	Pos	Pos	100
3	Neg	Neg	W. Pos	Neg	Pos	Pos	83
4	Neg	Neg	Pos	Pos	Pos	Pos	100
5	Neg	Neg	Pos	Pos	Pos	Pos	100
6.2	Neg	Neg	Pos	Pos	Pos	Pos	100
6.3	Neg	Neg	Pos	Pos	Pos	Pos	100
7	Neg	Neg	Neg	Neg	Pos	Pos	67
8	Neg	Neg	Pos	Pos	Pos	Pos	100
11	Neg	Neg	Pos	Pos	Pos	Pos	100
12.4	Neg	Neg	W. Pos	W. Pos	Pos	Pos	100
12.6	Neg	Neg	Neg	Neg	W. Pos	W. Pos	67
13	Neg	Neg	Neg	Neg	Pos	Pos	67
14	Neg	Neg	Pos	Pos	Pos	Pos	100
15	Neg	Neg	W. Pos	W. Pos	Pos	Pos	100
17	Neg	Neg	Pos	Pos	Pos	Pos	100
19	Neg	Neg	Pos	Pos	Pos	Pos	100
20	Neg	Neg	Pos	Pos	Pos	Pos	100
22	Neg	Neg	Pos	Pos	Pos	Pos	100
23	Neg	Neg	W. Pos	W. Pos	Pos	Pos	100
25	Neg	Neg	Pos	Pos	Pos	Pos	100
26	Neg	Neg	Neg	Neg	Pos	Pos	67
27	Neg	Neg	Pos	Pos	Pos	Pos	100
31	Neg	Neg	Neg	Neg	Pos	Pos	67
32	Neg	Neg	Neg	Neg	Pos	Pos	67
34.4	Neg	Neg	Pos	Pos	Pos	Pos	100
34.51	Neg	Neg	Pos	Pos	Pos	Pos	100
34.52	Neg	Neg	Pos	Pos	Pos	Pos	100
36	Neg	Neg	W. Pos	W. Pos	Pos	Pos	100
39	Neg	Neg	W. Pos	W. Pos	Pos	Pos	100
40	Neg	Neg	Pos	Pos	Pos	Pos	100
43	Neg	Neg	Neg	Neg	Pos	Pos	67
44	Neg	Neg	Neg	W. Pos	Pos	Pos	83
46	Neg	Neg	Neg	Neg	Pos	Pos	67
47	Neg	Neg	W. Pos	W. Pos	Pos	Pos	100
51	Neg	Neg	W. Pos	W. Pos	Pos	Pos	100
52	Neg	Neg	W. Pos.	Neg	Pos	Pos	83
53	Neg	Neg	Neg	Neg	Pos	Pos	67
54	Neg	Neg	Pos	Pos	Pos	Pos	100
56.31	Neg	Neg	Pos	Pos	Pos	Pos	100
56.32	Neg	Neg	W. Pos	W. Pos	Pos	Pos	100
58	Neg	Neg	Pos	Pos	Pos	Pos	100
59	Neg	Neg	Pos	Pos	Pos	Pos	100

TABLE I (cont'd)

Sample Nos. / Lab. Nos.	SB-1-74	SB-2-74	SB-3-74	SB-4-74	SB-5-74	SB-6-74	Score
61	Neg	Neg	Pos	Pos	Pos	Pos	100
62	Neg	Neg	Pos	Pos	Pos	Pos	100
63	Neg	Neg	Neg	W. Pos	Pos	Pos	83
64	Neg	Neg	Pos	Pos	Pos	Pos	100
65	Neg	Neg	Neg	Neg	Pos	Pos	67
70	Neg	Neg	Pos	Pos	Pos	Pos	100
71	Neg	Neg	Neg	Neg	Pos	Pos	67
72	Neg	Neg	Neg	Neg	Pos	Pos	67
						$\bar{x}$ =	90.9

* Request for repeat of questionable results were tabulated as W. pos.

Your laboratory code number is _____.

Appendix I

STATE OF UTAH—DEPARTMENT OF SOCIAL SERVICES

CALVIN L. RAMPTON
Governor

PAUL B. ROSE
Executive Director

DIVISION OF HEALTH
44 MEDICAL DRIVE
SALT LAKE CITY, UTAH 84113
AREA CODE 801
328-6131

Board of Health
Air Conservation Committee
Health Facilities Council
Medical Examiner Committee
Nursing Home Advisory Committee
Water Pollution Committee

LYMAN J. OLSEN, M.D., M.P.H.
Director of Health

BUREAU OF LABORATORIES

April 3, 1974

MEMORANDUM

TO: Participants, Immunology Proficiency Testing Program and Infectious Mononucleosis Test Evaluation Program

FROM: Bureau of Laboratories, Laboratory Improvement Program

SUBJECT: I. M. Test Results - Shipment No. 2, 1974

On March 4, 1974 six infectious mononucleosis test evaluation and proficiency testing specimens were shipped to participating laboratories. Table I shows the results obtained by each laboratory and their score.

The target values for these samples were as follows:

Specimen No.	Slide Tests	Ox-Cell Hemolysin (CDC Method)	Davidsohn Differential		
			Unabs.	G.P.K. Abs.	R.E. Abs.
SB-7-74 and SB-9-74	Neg	< 10	< 7	< 7	< 7
SB-8-74 and SB-10-74	Pos	80	56	≥ 7	< 7
SB-11-74 and SB-12-74	Pos	320	224	≥ 28	< 7

For state approval in immunology laboratories are required to obtain at least 75% agreement with referee laboratories. In this set of specimens the referee laboratories (Lab. Nos. 70, 71, and 72) received an average score of 72.3%. Therefore, the minimum acceptable score for state approval on this shipment is 75% of 72.3% or 54.2%. All laboratories except one received acceptable scores due to the repeated poor performance of the referee laboratories. Of the 53 test reports returned, 35 received scores of 100%, 7 received scores of 83%, 10 received scores of 67% and 1 received a score of 33%.

This shipment of specimens was a repeat of the first shipment with changed sequence and numbering. Specimen numbers SB-1-74, SB-2-74, SB-7-74 and SB-9-74 are all the same pools. Specimen numbers SB-3-74, SB-4-74, SB-8-74 and SB-10-74

are all the same pools and specimen numbers SE-5-74, SB-6-74, SE-11-74 and SB-12-74 are all the same pools. Laboratories should compare their results on these three pools to obtain an estimate of within laboratory reproducibility.

Analysis of results for the first two shipments revealed the following:

Test	No. of Reports Received	$\bar{x}$ Score
Monotest (Wampole)	40	90.0
Monosticon (Organon)	19	96.6
I. M. Kit (Micro. Sci.)	12	90.2
Monospot (Ortho)	11	93.9
Diagluto I. M. (Beckman)	8	85.5
Ox-Cell Hemolysin	6	66.8
Davidsohn Differential	5	86.6
Bacto-Hetrol (Difco)	2	100.0
Monosticon Dri-Dot (Organon)	2	100.0
	105	90.2

Some laboratories consistently do well while others consistently do poorly with the same tests. We are attempting to determine reasons for these differences and hope that the results will help laboratories improve their performance.

Your participation in this program is appreciated. If there are any questions concerning the program or your results please contact us.

Sincerely,

John L. Clayton, Microbiologist

Roger N. Taylor, Microbiologist

JLC/RNT/eh

TABLE I

Immunology Proficiency Testing and Infectious Mononucleosis Test Evaluation Results

Sample Nos.	SB-7-74	SB-8-74	SB-9-74	SB-10-74	SB-11-74	SB-12-74	
Lab. Nos.							Score
1.3	neg	pos	neg	pos	pos	pos	100 %
2.3	neg	w. pos	neg	w. pos	pos	pos	100
3.7	neg	w. pos	neg	w. pos	pos	pos	100
4.5	neg	pos	neg	pos	pos	pos	100
5.4	neg	pos	neg	neg	pos	pos	83
6.2	neg	neg	neg	pos	pos	pos	83
6.3	neg	neg	neg	pos	pos	pos	83
7.3	neg	pos	neg	pos	pos	pos	100
8.8	neg	pos	neg	pos	pos	pos	100
10.5	neg	neg	neg	neg	pos	pos	67
11.81	neg	neg	neg	pos	pos	pos	83
11.82	neg	neg	neg	pos	pos	pos	83
12.6	neg	neg(?)	neg	neg(?)	pos(?)	pos(?)	67
12.8	neg	w. pos	neg	w. pos	pos	pos	100
13.3	neg	neg	neg	neg	pos	pos	67
14.4	neg	pos	neg	pos	pos	pos	100
15.3	w. pos	neg	w. pos	neg	pos	pos	83
16.3	neg	pos	neg	pos	pos	pos	100
17.3	neg	neg	neg	w. pos	pos	pos	83
19.5	neg	pos	neg(?)	pos	pos	pos	100
20.9	neg	pos	neg	pos	pos	pos	100
22.3	neg	pos	neg	pos	pos	pos	100
23.7	neg	w. pos	neg	w. pos	pos	pos	100
25.5	neg	pos	neg	pos	pos	pos	100
26.7	neg	neg	neg	neg	pos	pos	67
27.3	neg	pos	neg	pos	pos	pos	100
31.7	neg	pos	neg	pos	pos	pos	100
32.3	neg	pos	neg	pos	pos	pos	100
33.3	neg	pos	neg	pos	pos	pos	100
34.2	neg	pos	neg	pos	pos	pos	100
34.4	neg	pos	neg	pos	pos	pos	100
39.3	neg	pos	neg	pos	pos	pos	100
40.1	neg	pos	neg	pos	pos	pos	100
43.4	neg	pos	neg	pos	pos	pos	100
44.8	neg	neg	neg	neg	pos	pos	67
46.5	neg	pos	neg	pos	pos	pos	100
51.5	neg	pos	neg	w. pos	pos	pos	100
52.5	neg	w. pos	neg	w. pos	pos	pos	100
52.8	neg	w. pos	neg	w. pos	pos	pos	100
53.3	neg	pos	neg	pos	pos	pos	100
54.5	neg	pos	neg	pos	pos	pos	100
55.3	neg	pos	neg	pos	pos	pos	100
56.32	neg	neg	neg	neg	pos	pos	67
58.8	neg	w. pos	neg	w. pos	pos	pos	100

TABLE I (cont'd)

Sample Nos.	SB-7-74	SB-8-74	SB-9-74	SB-10-74	SB-11-74	SB-12-74	
Lab. Nos.							Score
59.5	neg	w. pos	neg	w. pos	pos	pos	100
61.3	neg	pos	neg	pos	pos	pos	100
62.3	neg	w. pos	neg	w. pos	pos	pos	100
63.8	neg	w. pos	neg	w. pos	pos	pos	100
64.4	neg	neg	neg	neg	pos	pos	67
65.3	neg	neg	neg	neg	pos	pos	67
70.6	neg	neg	neg	neg	pos	pos	67
71.6	neg	neg	neg	neg	pos	pos	67
72.2	neg	pos	neg	neg	pos	pos	83
							90.7

Your laboratory code number is _____.

Appendix J

STATE OF UTAH–DEPARTMENT OF SOCIAL SERVICES

CALVIN L. RAMPTON
Governor

PAUL S. ROSE
Executive Director

DIVISION OF HEALTH
44 MEDICAL DRIVE
SALT LAKE CITY, UTAH 84113
AREA CODE 801
PHONE 328-6131

Board of Health
Air Conservation Committee
Health Facilities Council
Medical Examiner Committee
Nursing Home Advisory Council
Water Pollution Committee

BUREAU OF LABORATORIES

LYMAN J. OLSEN, M.D., M.P.H.
Director of Health

June 24, 1974

MEMORANDUM

TO: Participants, Immunology Proficiency Testing Program and Infectious Mononucleosis Test Evaluation Program

FROM: Bureau of Laboratories, Laboratory Improvement Program

SUBJECT: I. M. Test Results - Shipment No. 3, 1974

On 6 May 1974 six infectious mononucleosis test evaluation and proficiency testing specimens were shipped to participating laboratories. Table I shows the results obtained by each laboratory and their score.

The target values for these samples were as follows:

Specimen No.	Slide Tests	Ox-Cell Hemolysin (CDC Method)	Davidsohn Differential		
			Unabs.	G.P.K. Abs.	B.E. Abs.
SB-13-74 and SB-15-74	Neg	< 10	< 7	< 7	< 7
SB-14-74 and SB-18-74	Neg	< 10	56	56	56
SB-16-74 and SB-17-74	Pos	160	112	$\geq$ 14	< 7

For state approval in immunology, laboratories are required to obtain at least 75% agreement with referee laboratories. In this set of specimens the referee laboratories (Lab. Nos. 71 and 72) received an average grade of 100%. Therefore, the minimum acceptable grade for state approval on this shipment is 75%. Seven of the 49 participating laboratories received the unacceptable score of 67%. Three laboratories received scores of 83%, with the remainder receiving scores of 100%.

Tabulation of errors by type of test used gave the following:

TEST	% OF TESTS	% OF ERRORS	RATIO %E/%T
Davidsohn	5.9%	0.0%	0.0
Mono Test	33.3%	18.8%	0.6
Monospot	17.6%	25.0%	1.4
Monosticon	13.7%	43.8%	3.2
Ox-Cell Hemolysin	3.9%	0.0%	0.0
Digluto	7.8%	0.0%	0.0
I. M. Kit	13.7%	0.0%	0.0
Dri-Dot	2.0%	12.5%	6.3
Bacto-Heterol	2.0%	0.0%	0.0

The ratio of percent error to percent of tests is an indication of how well the test performed on this set of specimens. Little weight should be given to the Dri-Dot results since this represents results on only one laboratory. X^2 analysis revealed that the results were highly significant with $p < .001$.

Fourteen of the 16 errors on this shipment were on specimens 14 and 18. These specimens were designed to give false results if no absorption or incomplete absorption procedures were used. Since the Monosticon test can be done either with or without absorption, some of the errors reported with this test may have been due to failure to perform the absorptions.

Sincerely,

John L. Clayton, Microbiologist

Roger N. Taylor, Microbiologist

JLC/RNT/eh

TABLE I

Immunology Proficiency Testing and Infectious Mononucleosis Test Evaluation Results

Sample Nos.	SB-13-74	SB-14-74	SB-15-74	SB-16-74	SB-17-74	SB-18-74	
Lab. Nos.							Score
01.	neg	neg	neg	pos	pos	neg	100%
02.	neg	neg	neg	pos	pos	neg	100%
03.	neg	neg	neg	pos	pos	neg	100%
04.	neg	pos	neg	pos	pos	pos	67%
05.	neg	neg	neg	pos	pos	neg	100%
06.3	neg	neg	neg	pos	pos	neg	100%
06.2	neg	neg	neg	pos	pos	neg	100%
07.	neg	pos	neg	pos	pos	pos	67%
08.	neg	neg	neg	pos	pos	neg	100%
09.	neg	neg	neg	pos	pos	neg	100%
10.	neg	pos	neg	pos	pos	neg	83%
11.	neg	neg	neg	pos	pos	neg	100%
12.4	neg	neg	neg	pos	pos	neg	100%
12.6	neg	neg	neg	w. pos	w. pos	neg	100%
13.	w. pos	neg	neg	pos	pos	neg	83%
14.	neg	neg	neg	pos	pos	neg	100%
15.	neg	neg	neg	pos	pos	neg	100%
16.	neg	neg	neg	pos	pos	neg	100%
17.	neg	neg	neg	pos	pos	neg	100%
19.	neg	pos	neg	pos	pos	pos	67%
20.	neg	pos	neg	pos	pos	pos	67%
21.	neg	neg	neg	pos	pos	neg	100%
22.	neg	neg	neg	pos	pos	neg	100%
23.	neg	neg	neg	pos	pos	neg	100%
25.	neg	pos	neg	pos	pos	pos	67%
26.	neg	neg	neg	pos	pos	neg	100%
27.	neg	neg	neg	pos	pos	neg	100%
31.	neg	neg	neg	pos	pos	neg	100%
32.	neg	neg	neg	pos	pos	neg	100%
33.	neg	neg	neg	pos	pos	neg	100%
34.2	neg	neg	neg	pos	pos	neg	100%
34.3	neg	neg	neg	pos	pos	neg	100%
36.	neg	neg	neg	pos	pos	neg	100%
39.	neg	neg	neg	pos	pos	neg	100%
40.	neg	neg	neg	pos	pos	neg	100%
43.	neg	neg	neg	pos	pos	neg	100%
44.	neg	neg	neg	pos	pos	neg	100%
46.	neg	pos	neg	pos	pos	neg	67%
51.	neg	w. pos	neg	pos	pos	w. pos	67%
52.41	neg	neg	neg	pos	pos	neg	100%
52.82	neg	neg	neg	pos	w. pos	neg	100%

TABLE I (cont'd)

Immunology Proficiency Testing and Infectious Mononucleosis Test Evaluation Results

Sample Nos.	SB-13-74	SB-14-74	SB-15-74	SB-16-74	SB-17-74	SB-18-74	
Lab. Nos.							Score
53.	neg	neg	neg	pos	pos	neg	100%
54.	neg	neg	neg	pos	pos	neg	100%
55.	neg	neg	neg	pos	pos	neg	100%
58.	neg	neg	neg	pos	pos	neg	100%
61.	neg	neg	neg	pos	pos	neg	100%
62.	neg	neg	neg	pos	pos	neg	100%
63.	neg	neg	neg	pos	pos	neg	100%
64.	neg	neg	?	pos	pos	neg	83%
71.	neg	neg	neg	pos	pos	neg	100%
72.	neg	neg	neg	pos	pos	neg	100%

$\bar{x}$ = 94.5%

Your laboratory code number is _____.

Appendix K

STATE OF UTAH—DEPARTMENT OF SOCIAL SERVICES

PAUL S. ROSE
Executive Director

DIVISION OF HEALTH
44 MEDICAL DRIVE
SALT LAKE CITY, UTAH 84113
AREA CODE 801

Board of Health
Air Conservation Committee
Health Facilities Council
Medical Examiner Committee
Nursing Home Administrators
Water Pollution Committee

LYMAN J. OLSEN, M.D., M.P.H.
Director of Health

328-6131

BUREAU OF LABORATORIES

August 1, 1974

MEMORANDUM

TO: Participants, Immunology Proficiency Testing Program and Infectious Mononucleosis Test Evaluation Program

FROM: Bureau of Laboratories, Laboratory Improvement Program

SUBJECT: I. M. Test Results - Shipment No. 4, 1974

On 8 July 1974 six infectious mononucleosis test evaluation and proficiency testing specimens were shipped to participating laboratories. Table I shows the results obtained by each laboratory and their score.

The target values for these samples were as follows:

Specimen No.	Slide Tests	Ox-Cell Hemolysin (CDC Method)	Davidsohn Differential		
			Unabs.	G.P.K. Abs.	B.E. Abs.
SB-20-74 and SB-21-74	neg	< 10	< 7	< 7	< 7
SB-19-74 and SB-24-74	neg	< 10	56	56	56
SB-22-74 and SB-23-74	pos	160	112	≥ 14	< 7

For state approval in immunology, laboratories are required to obtain at least 75% agreement with referee laboratories. In this set of specimens the referee laboratories (Lab. Nos. 71 and 72) received an average grade of 100%. Therefore, the minimum acceptable grade for state approval on this shipment is 75%. Four of the 52 participating laboratories received the unacceptable score of 67% and one received a score of 50%. Two laboratories received scores of 83%, with the remainder receiving scores of 100%.

We are in the process of analyzing the sensitivity, specificity and reproducibility of the various tests in use within the state and hope to be able to report these

results to participating laboratories in the near future. We are also analyzing factors which may affect test performance and will include these results in the report.

Your cooperation in this project has been appreciated.

Sincerely,

John L. Clayton, Microbiologist

Roger N. Taylor, Microbiologist

RNT/JLC/eh

TABLE I

Immunology Proficiency Testing and Infectious Mononucleosis Test Evaluation Results

Sample Nos.	SB-19-74	SB-20-74	SB-21-74	SB-22-74	SB-23-74	SB-24-74	
Lab. Nos.							Score
01	neg	neg	neg	pos	pos	neg	100%
02	neg	neg	neg	pos	pos	neg	100
03	neg	neg	neg	pos	pos	neg	100
04	pos	neg	neg	pos	pos	neg	83
05	neg	neg	neg	w. pos	pos	neg	100
06.2	neg	neg	neg	pos	pos	neg	100
06.3	neg	neg	neg	pos	pos	neg	100
07	neg	neg	neg	pos	pos	neg	100
08	neg	neg	neg	pos	pos	neg	100
09	neg	neg	neg	pos	pos	neg	100
10	neg	neg	neg	pos	pos	neg	100
11	neg	neg	neg	pos	pos	neg	100
12.6	w. pos	neg	neg	pos	pos	w. pos	67
12.8	w. pos	neg	neg	pos	pos	w. pos	67
13	neg	neg	neg	pos	pos	neg	100
14	neg	neg	neg	pos	pos	neg	100
15	neg	neg	neg	pos	pos	neg	100
16	neg	neg	neg	pos	pos	neg	100
17	neg	neg	neg	pos	pos	neg	100
19	neg	neg	neg	pos	pos	neg	100
20	neg	neg	neg	pos	pos	neg	100
21	neg	neg	neg	pos	pos	neg	100
22	neg	neg	neg	pos	pos	neg	100
23	neg	neg	neg	pos	pos	neg	100
25	neg	neg	neg	pos	pos	neg	100
26	neg	neg	neh	pos	pos	neg	100
27	neg	pos	pos	pos	pos	pos	50
31	neg	neg	neg	pos	pos	neg	100
32	neg	neg	neg	pos	pos	neg	100
33	neg	neg	neg	pos	pos	neg	100
34.2	neg	neg	neg	pos	pos	neg	100
34.8	neg	neg	neg	pos	pos	neg	100
36	neg	neg	neg	w. pos	pos	neg	100
39	neg	neg	neg	pos	pos	neg	100
40	neg	neg	neg	pos	pos	neg	100
43	neg	neg	neg	pos	pos	neg	100
44	neg	neg	neg	pos	pos	neg	100
46	neg	neg	neg	pos	pos	neg	100
51	pos	neg	neg	pos	pos	pos	67
52.4	neg	neg	neg	pos	pos	neg	100
52.9	neg	neg	neg	pos	pos	neg	100

TABLE I (cont'd)

Immunology Proficiency Testing and Infectious Mononucleosis Test Evaluation Results

Sample Nos.	SB-19-74	SB-20-74	SB-21-74	SB-22-74	SB-23-74	SB-24-74	
Lab. Nos.							Score
53	neg	neg	neg	pos	pos	neg	100%
54	neg	neg	neg	pos	pos	neg	100
55	neg	neg	neg	pos	pos	neg	100
56	neg	neg	neg	pos	pos	neg	100
58	neg	neg	neg	neg	pos	neg	83
59	w. pos	neg	neg	pos	pos	w. pos	67
61	neg	neg	neg	pow	pos	neg	100
62	neg	neg	neg	pos	pos	neg	100
63	neg	neg	neg	w. pos	w. pos	neg	100
64	neg	neg	neg	pos	pos	neg	100
65	neg	neg	neg	pos	pos	neg	100
71	neg	neg	neg	pos	pos	neg	100
72	neg	neg	neg	pos	pos	neg	100
						$\bar{x}$	96.0

Your laboratory code no. is _____

Appendix L

STATE OF UTAH—DEPARTMENT OF SOCIAL SERVICES

CALVIN L. RAMPTON
Governor

PAUL S. ROSE
Executive Director

DIVISION OF HEALTH
44 MEDICAL DRIVE
SALT LAKE CITY, UTAH 84113
AREA CODE 801
PHONE 328-6131

Board of Health
Air Conservation Committee
Health Facilities Council
Medical Examiner Committee
Nursing Home Advisory Council
Water Pollution Committee

BUREAU OF LABORATORIES

LYMAN J. OLSEN, M.D., M.P.H.
Director of Health

October 18, 1974

MEMORANDUM

TO: Participants, Immunology Proficiency Testing Program and Infectious Mononucleosis Test Evaluation Survey

FROM: Bureau of Laboratories, Laboratory Improvement Program

SUBJECT: I. M. Test Evaluation Report

During the year the Utah State Division of Health has been conducting an evaluation of tests for infectious mononucleosis in conjunction with its immunology proficiency testing program. This report contains a brief summary of the findings obtained by means of this evaluation. If there are any questions or if more information is desired please contact the Bureau of Laboratories, Laboratory Improvement Program.

Table I shows the evaluation of results by type of test indicating the sensitivity, specificity, reproducibility and agreement achieved by each test.

Monotest (Wampole) was by far the most frequently used test in this evaluation (27/88 or 30.7%) with Monospot (Ortho) (13/88 or 14.8%), Monosticon (Organon) (10/88 or 11.4%), I. M. Kit (Micro. Resh. Corp.) (10/88 or 11.4%), Diagluto I. M. (Beckman) (8/88 or 9.1%) and Monosticon Dri-Dot (Organon) (7/88 or 8.0%) following.

Of the tests used by three or more laboratories the most sensitive was Monosticon Dri-Dot (Organon) (100%) and the least sensitive was the ox-cell hemolysin (68.8%). The Davidsohn differential and Diagluto I.M. (Beckman) both achieved 100% specificity with Monosticon (Organon) and Monositcon Dri-Dot (Organon) receiving the lowest specificity (87.0% and 86.6% respectively). There was an inversely proportional relationship between sensitivity and specificity ($P < .02$), i.e., as sensitivity increased specificity decreased.

Within shipment reproducibility was consistantly about 5 percentage points higher than the between shipment reproducibility ($P << .001$).

The Davidsohn differential obtained the best agreement with target values but it was not significantly better than the slide tests. None of the differences in tests were significant.

Although there were a number of changes in test use, frequently multiple changes, eighty percent of the laboratories did not change tests during the evaluation.

Performance improved during the evaluation as revealed by the fact that agreement was 5 percentage points higher for the second half than it was for the first half ($P < .05$). Presumably this was attributable to increased proficiency which resulted from the educational and corrective influence of proficiency testing.

Private hospitals achieved significantly lower sensitivity ($P < .01$) and agreement ($P < .05$) than other types of laboratory. Sensitivity was 69.0% versus 93.3% and the agreement was 82.2% versus 92.8% respectively. Thus, private hospitals are more likely to miss a case of infectious mononucleosis.

Technologists with a baccalaureate degree achieved a little better agreement (92.8%) than did either the high school graduates (90.0%) or the technologists with masters degrees (89.8%) but the differences were not statistically significant. The one "technologist" with a doctorate did very well (100% agreement, specificity and sensitivity) but his performance was not significantly better than any other group.

The two I.S.C.L.T. registered technologists were about 10 percentage points lower in agreement (79.5% vs. 92.8%) than the other groups (A.S.C.P., A.M.T. and non-registered technologists). Sensitivity was also lower for this group (66.5% vs. 92.1%).

Experience of the technologist made little difference, but in general the group with 3 to 10 years of experience performed the best, followed by the group with 0 to 2 years, and the group with more than 10 years experience did the poorest. Experience with the particular test being used had about the same results. Technologists with 3 or more years of experience with the particular test had significantly lower ($P < .05$) sensitivity (85.2% vs. 93.8%) than the other groups. Apparently, experience increases performance up to a point after which performance deteriorates to a level lower than those with no experience.

Most laboratories (54.5%) used a procedure which did not involve any absorption, and most of the laboratories (75%) which used an absorption procedure used both guinea pig kidney and beef erythrocytes. There were no significant differences among the various absorption procedures.

There were no significant differences among the different agglutination end points and the significant differences in the 50 hemolytic end point was probably not due to the test itself.

Both the length of time the specimens were held before testing and the temperature at which they were held had an effect on the reliability of the test. Specificity, reproducibility, and agreement were all lower for specimens held over one week before testing than they were for those tested within one

week. Sensitivity, specificity and agreement were all lower for specimens held at room temperature than they were for specimens held at refrigerator temperatures, but reproducibility was higher when specimens were held at room temperature. When the combined effects of time and temperature were evaluated it was observed that all of the parameters were 2 to 6 percentage points lower for the specimens which were held the longest times and the highest temperatures.

We were unable to evaluate the effect of controls since 96% of the laboratories used at least a positive control.

Most laboratories (52.4%) used the same test as both screen and confirmation and many laboratories (41.5%) used the tests only as screens. There were no significant differences in tests by use.

Most laboratories (79.8%) used tests in which the technologists had "good" confidence and none of the laboratories used tests in which the technologists had "poor" confidence. Although none of the differences were significant the tests in which technologists had only "fair" confidence performed slightly better in general than did the tests in which the technologist expressed "good" confidence.

In general, technologists specializing in hematology or blood banking obtained the best results, with bacteriologists and serologists next, and chemists and general medical technologists obtained the poorest results.

Beef cells obtained significantly lower ($P < .05$) agreement (79.2% vs. 93.1%) than fresh sheep cells or the three groups of horse cells. The beef cells were also less sensitive ($P < .01$) than the other cells (68.8% vs. 92.6%), but this was probably due to evaluation problems rather than inadequacy of the cells or the test.

As the number of serologic tests performed annually in the laboratory increased the performance increased although some of the differences were not significant. Similar results were observed with the number of tests for infectious mononucleosis performed in the laboratory, but there was a point after which performance declined.

One-man laboratories achieved significantly higher ($P < .05$) total reproducibility (98.0% vs. 93.3%) than other laboratories. Agreement increased as the number of technicians increased, with the laboratory with more than five technicians achieving significantly better ($P < .05$) agreement (95.5% vs. 91.7%) than laboratories with only one technician.

Table II whows the reported costs by type of test. There were no significant differences between the reported cost and time requirements for the two standard test, nor among the slide tests. The standard tests required 30.0 ± 7.1 minutes to perform and cost $\$2.48 \pm .14$ for cost of materials and technologist's tire. The slide tests required 6.5 ± 2.6 minutes to perform and cost $\$1.46 \pm .44$ for materials and technologist's time.

Subjective evaluation of the tests showed that speed and ease of performance were the most frequently mentioned advantages and cost and lack of titer were the most frequently mentioned disadvantages.

It seems that a small laboratory could do satisfactory testing for infectious mononucleosis by selecting one of the slide tests that meet their requirements for cost, shelf life, titer capability, etc., and using it properly. For larger laboratories the slide tests would serve well as screens with the standard tests (Davidsohn differential or ox-cell hemolysin) used for titration when they are requested or for confirmation.

Even though most of the parameters measured and variables evaluated exhibited substantial differences they were usually not significantly different due to the small sample size in some cases and large variation in most cases. This points out the need for similar evaluations using larger proficiency testing programs. Similar programs should also be conducted to evaluate other types of tests so that test selection can be made on the basis of measured effectiveness rather than the methods currently used.

Sincerely,

Roger N. Taylor, Microbiologist
Laboratory Improvement Program

RNT/eh

TABLE I

Evaluation of Results by Type of Test

Test	No.*	% of total	Sensitivity	Specificity	Reproducibility			Agreement
					Within	Between	Total	
Bacto-Hetrol (Difco)	2	2.3	100.0	100.0	100.0	100.0	100.0	100.0
Davidsohn Differential	4	4.6	91.8	100.0	96.0	92.0	96.0	96.0
Monotest (Wampole)	27	30.7	90.7	96.3	99.0	89.4	94.6	93.0
Monospot (Ortho)	13	14.8	95.5	94.2	97.5	91.2	95.6	94.3
Monosticon (Organon)	10	11.4	92.6	87.0	96.3	91.4	95.9	88.3
Ox-Cell Hemolysin	4	4.6	68.8	95.8	100.0	83.5	89.0	79.2
Diagluto (Beckman)	8	9.1	84.4	100.0	97.9	94.3	96.5	90.6
I.M. Kit (MicroResCorp)	10	11.4	91.7	96.7	95.1	91.6	93.9	92.2
Dri-Dot (Organon)	7	8.0	100.0	86.6	96.0	89.5	94.7	93.3
Mono-Diff (Wampole)	1	1.1	100.0	100.0	100.0	---	100.0	100.0
Monophile (Bio-Diagnostic Systems)	1	1.1	100.0	100.0	100.0	---	100.0	100.0
Rythrotex (BMC)	1	1.1	100.0	100.0	100.0	---	100.0	100.0
Total	88	100.0	92.4	94.8	97.6	90.4	95.1	92.4

* Indicates number of laboratory-technologist combinations (some laboratories had more than one technologist reporting or more than one test used.

TABLE II

Reported Costs by Type of Test

Test	Time (min)	Time cost ($)	Material cost ($)	Total cost ($)	No. of reports
Bacto-Hetrol (Difco)	12.5 ± 2.9	1.08 ± .38	.60 ± .49	1.68 ± .36	5
Davidsohn Differential	35.0 ± 8.7	2.12 ± .12	.46 ± .05	2.58 ± .11	3
Monotest (Wampole)	7.0 ± 5.8	0.60 ± .49	.47 ± .24	1.07 ± .53	24
Monospot (Ortho)	6.7 ± 2.5	0.43 ± .17	1.36 ± .81	1.70 ± .60	9
Monosticon (Organon)	6.6 ± 3.1	0.46 ± .20	1.48 ± 1.11	1.94 ± .82	10
Ox-Cell Hemolysin	25.0 ± 17.3	1.75 ± .43	.63 ± .29	2.38 ± .72	3
Diagluto (Beckman)	7.8 ± 4.7	0.49 ± .31	.70 ± .56	1.38 ± .45	5
I.M. Kit (MicroRshCorp)	5.2 ± 2.1	0.58 ± .39	.61 ± .28	1.19 ± .58	9
Dri-Dot (Organon)	6.4 ± 3.4	0.61 ± .32	.99 ± .40	1.60 ± .40	7
Mono-Diff (Wampole)	5.0 ± 0	0.25 ± 0	2.00 ± 0	2.25 ± 0	1
Monophile (Bio-Diag-Sys)	2.0 ± 0	0.14 ± 0	1.00 ± 0	1.14 ± 0	1
Rythrotex (BMC)	6.0 ± 0	0.42 ± 0	.37 ± 0	.79 ± 0	1
$\bar{x}$ ± SD	10.4 ± 9.7	0.74 ± .61	.89 ± .50	1.61 ± .55	78
Tube test total	30.0 ± 7.1	1.94 ± .26	.54 ± .12	2.48 ± .14	2
Slide test total	6.5 ± 2.6	.51 ± .25	.96 ± .52	1.46 ± .44	10
Total	10.4 ± 9.7	0.74 ± .61	.89 ± .50	1.61 ± .55	12

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VITA

Name	Roger Norris Taylor
Birthplace	Farmington, Utah
Birthdate	23 October 1941
University	University of Utah Salt Lake City, Utah 1959-1974
Degrees	B.S., University of Utah Salt Lake City, Utah 1969
	M.S., University of Utah Salt Lake City, Utah 1971
	Ph.D., University of Utah Salt Lake City, Utah 1974
Professional Organizations	American Society for Microbiology 1969-1974
	American Association for the Advancement of Science 1970-1974
	Federation of American Scientists 1973-1974
	Utah Public Health Association 1974
	American Academy of Microbiology (Specialist in Public Health and Medical Laboratory Microbiology) (Pending)

Professional Positions

Research Microbiologist
Microbiology Research
Veterans Administration Hospital
Salt Lake City, Utah
1969-1971

Clinical Microbiologist
Clinical Laboratory
Veterans Administration Hospital
Salt Lake City, Utah
1971

Research Microbiologist
Infectious Disease Research
Veterans Administration Hospital
Salt Lake City, Utah
1971-1972

Microbiologist
Laboratory Improvement
Utah State Division of Health
Salt Lake City, Utah
1972-1974

Teaching Assistant
Department of Microbiology
College of Medicine
University of Utah
Salt Lake City, Utah
1969-1974