WHITE MOUNTAIN RESEARCH STATION

UNIVERSITY OF CALIFORNIA, BERKELEY

FINAL REPORT

ON SUBCONTRACT Z-847905-G

BETWEEN

BECKMAN INSTRUMENTS, INCORPORATED

AND

THE REGENTS OF THE UNIVERSITY OF CALIFORNIA

"TECHNICAL SUPPORT FOR ELECTROPHORETIC BLOODSTAIN ANALYSIS PROGRAM

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INTRODUCTION

The first suggestion that human beings could be differentiated by means of their blood came in 1900 when Karl Landsteiner discovered the first known blood types. Since that time, geneticists have made major contributions to our knowledge of the individualizing characteristics of fresh blood and to our techniques of blood analysis. These momentous discoveries were quickly put to use in the field of clinical medicine, and methodology was standardized for routine blood analysis in the clinical laboratory. However, the forensic scientist has been remarkably slow in utilizing these findings and adapting this methodology to the needs of the crime laboratory.

The National Institute of Law Enforcement and Criminal Justice, Law Enforcement Assistance Administration (LEAA), has undertaken to raise the quality and availability of forensic services in this country. This agency contracted with The Aerospace Corporation to assess the state of the art of forensic blood analysis for identification purposes in the United States, to define the problems to be overcome in increasing the use of blood analysis techniques in crime laboratories, and to identify promising approaches toward the solution of these problems. In April, 1974, The Aerospace Corporation issued a report (1) on its survey and assessment of blood and bloodstain analysis in American crime laboratories. Reference will again be made to the findings and recommendations of this Aerospace report.

Having determined some of the problems, The Aerospace Corporation, sponsored by LEAA, undertook a program for the development of methodology and equipment for blood and bloodstain analysis which would meet the particular needs of the U.S. crime laboratory.

In October of 1973, The Aerospace Corporation published a solicitation for research and development sources for a blood analysis methodology. Beckman Instruments Inc., Advanced Technology Operations (ATO) responded with a Letter of Interest, stating that Beckman ATO, in cooperation with this University laboratory, "offered unparalleled program capability in the technologies appropriate to the problem areas". In April of 1974, Beckman ATO submitted a proposal "Bloodstain Analysis Program" to the Aerospace Corporation. This proposal, written with the technical assistance of Dr. Benjamin W. Grunbaum of the White Mountain Research Station (WMRS) of the University of California, proposed that the development of methodology for the analysis of protein and enzyme systems in bloodstains be carried out at the WMRS under a major subcontract with the University of California. Ultimately the Aerospace contract was awarded to Mason Research Institute. However, Aerospace Corporation terminated all work on this project prior to the scheduled Feasibility Verification Demonstration.

In June of 1976, The Aerospace Corporation issued a Statement of Work for a Bloodstain Analysis System (2) with a subject request for proposal.

Again with the assistance of Dr. Grunbaum, Beckman responded with a proposal for "An Electrophoretic Bloodstain Analysis Program". At the request of The Aerospace Corporation, this proposal was clarified (October, 1976) to specify that Dr. Grunbaum would be key investigator in a subcontract with the University of California. Subsequently, The Aerospace Corporation awarded Beckman a subcontract[1] to provide the necessary personnel, services, materials,

[1] Aerospace Subcontract W-67854 to Beckman ATO.

and facilities to complete the tasks and subtasks described in that Statement of Work. Through a subcontract[2] awarded to the Regents of the University of California, WMRS entered into an agreement to give technical support in the development of this Bloodstain Analysis System (BAS) with Dr. Grunbaum and Professor Nello Pace acting as Co-Principal Investigators and Dr. Grunbaum designated as Project Director for the University.

The technical requirements for the proposed BAS were inspired by the earlier Aerospace survey (1) of the needs of American crime laboratories for improved blood analysis methodology. With the information available at the time the University accepted the subcontract, these technical requirements seemed both feasible and desirable. Furthermore, there was assurance that all interested parties fully understood the nature of the research and development to be undertaken[3]. There was realistic acceptance of the fact that new data and unexpected results might necessitate modification in the basic program plan, and provisions were made to implement such revisions "in a timely manner to preclude inefficient use of manpower and unfavorable cost variances associated with such inefficiencies."[3]

The work began in January, 1977, and progressed with highly satisfactory laboratory results. Understanding and community of purpose was promoted in large measure through the efforts and administrative skills of Mr. G. Roberts and R. Kennel of The Aerospace Corporation.

[2] Beckman Subcontract Z-847905-G to University of California, WMRS.

[3] "10.1.1. Program Plan Revisions (SOW 5.1.1.). It is probable that the Program Plan will require modification during the course of the investigation as new data and unexpected results are obtained. The internal program review process will guarantee the timely recognition of events and factors which mandate or make attractive revision of the basic Program Plan. As such circumstances are recognized, proposed revisions to the Program Plan will be submitted to the Aerospace Corporation for approval. These proposed revisions will be submitted to The Aerospace Corporation in a timely manner to preclude inefficient use of manpower and unfavorable cost variances associated with such inefficiencies."
Beckman Instruments, Inc., ELECTROPHORETIC BLOODSTAIN ANALYSIS PROGRAM PLAN, p. 15. (3)

The U.C. subcontract did not require nor provide in the budget for periodic written reports from WMRS concerning research progress and recommendations. Nevertheless, through May of this year, there seemed to be a good understanding of laboratory developments on the part of both Beckman ATO and The Aerospace Corporation, and there seemed to be agreement in regard to basic approaches. Frequent visits to the WMRS by the Beckman Program Manager and regular Program Review Meetings in Berkeley, including key personnel of the three concerned organizations, seemed to provide opportunity for adequate and accurate flow of information from this laboratory. The first five Monthly Progress Reports[4] from Beckman to The Aerospace Corporation and the minutes of the first three Program Review Meetings[5], prepared by Aerospace, show a reasonable accuracy and understanding concerning research-in-progress.

In June, the whole character of the contractual arrangement deteriorated, perhaps only coincidently when The Aerospace Corporation suddenly and without explanation changed management of this project and a person new to Aerospace took over as Program Manager.

The first evidence of a serious failure in communication came with the minutes of the fourth Program Review Meeting[5], June 20, 1977. These minutes, prepared by the Aerospace Program Manager, report certain research findings, conclusions, and "decisions" that are reflected in neither the May nor the June Progress Reports from Beckman ATO. The Project Director from WMRS was not present at the fourth Program Review Meeting and the University of California had no official representation. The individuals to whom these remarks are attributed were not authorized to speak for the WMRS

[4] Appendix, Section C.

[5] Appendix, Section D.

and their authority as decision-makers is not specified in any subcontract or program plans. Dr. Grunbaum subsequently protested[6] to Beckman ATO as to both the impropriety and inaccuracy of the observations and conclusions reported in these minutes. These comments were not acknowledged, nor were the minutes changed as requested.

Misrepresentation and misinterpretation of laboratory results appeared in subsequent Aerospace official minutes of Program Review Meetings. Requests from the Project Director to Beckman for revision of the errors were not effective. Research findings and recommendations from this laboratory were largely ignored.

In an effort to re-establish communication with Beckman ATO and force a realistic evaluation of research results, the Project Director at WMRS submitted three unsolicited progress reports[7] to Beckman ATO, dated 10 January to 31 July 1977, 1-31 August 1977, and 1-30 September 1977. These reports were not acknowledged, nor were their contents discussed and evaluated with the Project Director at WMRS. There is no indication that the contents of these reports were made known to The Aerospace Corporation. The same information was reported at Program Review Meetings in August and October, 1977, but was not reflected in the minutes of the meetings[8].

In spite of these difficulties, the developmental work in the WMRS laboratory remained on schedule through the Systems Definition phase of the project, which ended on September 1, 1977.

[6] APPENDIX, Section B. See letter to J. Walsh, 20 July 1977, and memo to J. Walsh, 10 August 1977.

[7] APPENDIX, Section A.

[8] APPENDIX, Section B. See letter to Jean Bordeaux, 24 October 1977; letter to Dr. B. W. Grunbaum, 27 October 1977; letter to Jean Boredeaux, 2 November 1977.

On September 6, prior to receipt of the Progress Report[9] from WMRS covering the work done in this laboratory during the month of August, and without consultation with the Project Director at WMRS, a letter[10] was sent from Beckman ATO to WMRS, strongly and very specifically stipulating the direction of future effort at system development in this laboratory. Since the decision was directly counter to the research findings and recommendations of the WMRS, this laboratory had the choice either of supporting an effort that appeared to be a waste of public funds, or to ask for release from further obligation in this development. It chose the latter course, and Beckman Instruments complied by rewriting the subcontract to terminate developmental work in this laboratory on November 15, 1977.

[9] APPENDIX, Section A.

[10] APPENDIX, Section B. Letter to B. Grunbaum, September 6, 1977.

BACKGROUND

In 1974, when the Aerospace Report (1) was published, it became apparent that relatively little bloodstain analysis was being done in leading American crime laboratories.

Aerospace had contacted 26 crime laboratories in the United States and one in Canada to determine their experience with blood analysis. Even among leading laboratories, the immunological blood group typings were primarily restricted to the ABO system. Relatively few laboratories were attempting any MN and Rh group analysis or analysis for enzyme/protein systems. The few laboratories attempting such analyses did so in only a minimal number of cases.

The Aerospace Report concluded that several factors contribute to the limited use of blood analysis in U.S. crime laboratories. These include 1) lack of funds, 2) insufficiently large staffs of trained forensic serologists, 3) lack of simple, rapid methods of analyses, 4) unavailability of antisera of sufficient quality for bloodstain analysis, and 5) lack of blood genetic marker frequency distribution data for the U.S. population.

There is every indication that this unfortunate low capability in dried bloodstain analysis in U.S. crime laboratories still exists today. LEAA recently publicized results of a federally-funded three-year project to test the capabilities of crime laboratories operated by federal, state, and local law enforcement agencies.[11] The release reported a wide range of error in response to tests including bloodstains. Only 40% of the laboratories tested gave correct answers on a bloodstain comparison test. The others made incomplete or inconclusive answers because they lacked the ability to perform

[11] U.S. Department of Justice, Law Enforcement Assistance Administration News Release, Washington, D.C., 8 April 1977. This study was conducted by the Forensic Sciences Foundation for LEAA.

more sophisticated tests or made mistakes in the tests. Less than 25% of the 232 laboratories did any additional grouping (Rh, MN, AK, EAP, PGM, Hb, Hp, Rheumatoid) beyond the ABO factor. Less than 18% attempted more than one additional analysis, and less than 3% attempted more than two analyses.

For several years prior to the 1974 survey, LEAA and several forensic societies attempted with little success to promote an increased use of bloodstain analysis in U.S. crime laboratories by co-sponsoring seminars and workshops given by B. J. Culliford of the London Metropolitan Police Laboratory. In 1971, LEAA published a volume (4) on bloodstain methodology by Culliford which was widely distributed to American criminalists concerned with forensic serology.

It is apparent from the results of both of the LEAA-sponsored surveys, one conducted by The Aerospace Corporation and the other by the Forensic Sciences Foundation, that American criminalists have not accepted the Culliford methodology. The Aerospace Report gives some reasons why this methodology has not been adapted in this country's crime laboratories. The British crime laboratories operate under the jurisdiction of a national police organization and thus it is possible to centralize blood analysis work in a few large forensic centers. There a staff of serologists are free to concentrate solely in blood analysis work.

In the United States, with a few exceptions, most crime laboratories are small and are operated under a variety of local jurisdictions. The volume of cases involving blood and bloodstain evidence is relatively small, so there is little justification to employ a specialist in serology. The American criminalist must be prepared to deal with a wide variety of physical evidence. When there are other heavy demands upon the time, resources and available

personnel of a given laboratory, its dried bloodstain analysis program may be very rudimentary. Current methodology is tedious and time-consuming, and the results may be imprecise; consequently, U.S. criminalists are not motivated to learn the necessary skills, request the necessary specialized equipment and supplies, and devote the necessary work time to bloodstain analysis.

The Aerospace Report, in its conclusions and recommendations, suggested that, if the present system of small local U.S. crime laboratories is to be continued, several actions are essential to promote more extensive bloodstain analysis, including "development of rapid and/or simultaneous electrophoretic separation and identification of several selected enzyme/protein systems"[12]. This stated need has led to the current Aerospace BAS Program.

The majority of the electrophoretic separations of enzymes and protein systems in forensic work are now performed on starch gel, with the exception of hemoglobin which is separated on cellulose acetate, and haptoglobin which is separated on polyacrylamide gradient gel. The gel methods require long run times, inconvenience, and possible error in the preparation of fresh substrate prior to each run, and considerable technical skill in performance. The American criminalist has found that the existing analytical methods are time-consuming and often yield ambiguous results.

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[12] The Aerospace Corporation SURVEY AND ASSESSMENT BLOOD AND BLOODSTAIN ANALYSIS PROGRAM, Vol. I, p. 134.

OBJECTIVES

The overall objective of the BAS Project was the development of methods, procedures, and reagents for an improved electrophoretic system of methodologies for the determination of enzyme and protein polymorphisms. Procedures and instrumentation must be suitable for utilization by small criminalistic laboratories. Specifically, the objectives of this program were as follows:

" ○ Reduce analyst skill requirements.

○ Reduce analysis time.

○ Reduce complexity in interpreting research results.

○ Be applicable to old stains.

○ Achieve discrimination probability of 1 out of 200."[13]

The technical requirements for the BAS are spelled out in Annex C of the Aerospace Statement of Work.

[13] The Aerospace Corporation, Statement of Work for a Bloodstain Analysis System.

RESEARCH MANAGEMENT-TECHNICAL SUPPORT

The Program Plan states that "Beckman plans to subcontract with the University of California, Berkeley, so that Dr. Grunbaum's talents can be applied to satisfying the objectives of the proposed program".[14] It further states that "Dr. Grunbaum will manage the work performed at the White Mountain Research Station including the technical efforts of the Consultants".[15] Figure 1[16] shows the organizational structure for the BAS Project.

Consultants

At Dr. Grunbaum's suggestion, the Beckman Proposal for a BAS included technical support by an expert in the electrophoretic methodology now in use in crime laboratories.

It was originally requested in the Beckman U.C. subcontract that Dr. Mathias Yoong of the Illinois Bureau of Investigation be engaged in support of the key investigators at WMRS. If for any reason Dr. Yoong was not available, then the University of California was to engage an individual of comparable background and experience acceptable to Beckman and the Aerospace Corporation to participate in this effort.

Dr. Yoong seemed an ideal choice because of his work, his experience in the crime laboratory, and his familiarity with diverse bloodstain methodologies, including those of the Metropolitan Police Laboratory in London. It was felt that Dr. Yoong, because of his scholarly training, would be able to play an active part in both the development of new methods and adaptation of existing methods for use in the BAS Project. Unfortunately, Dr. Yoong was unable for personal reasons to accept a short-term offer of this nature.

[14] Revised Program Plan, p. 8.
[15] Ibid., p. 9.
[16] Ibid., p. 7.

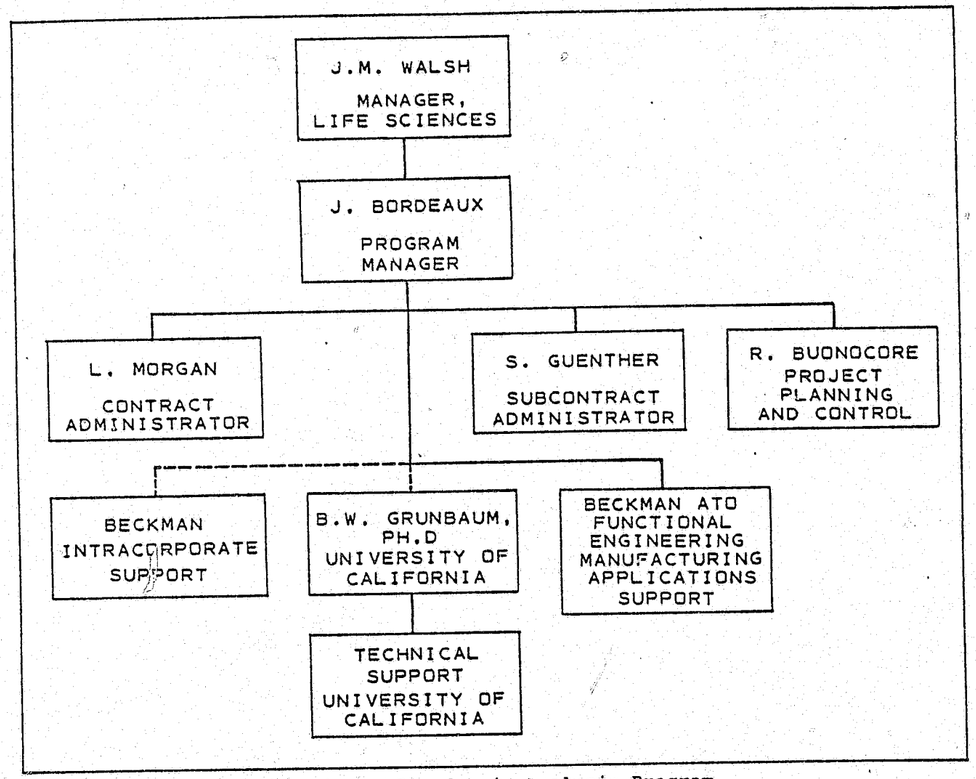

Figure 1. Organization Chart--Bloodstain Analysis Program

From: ELECTROPHORETIC BLOODSTAIN ANALYSIS PROGRAM
PROGRAM PLAN
Subcontract No. W-67854
Beckman Project 1361-2700-800
Dated: February 11, 1977
Submitted by Beckman Instruments, Inc., Advanced Technology Operations, to The Aerospace Corporation, Washington, D.C. 20024.

It was possible to secure the services of Mr. Brian Wraxall of the Metropolitan Police Laboratory in London and Mr. Mark Stolorow from the Michigan State Police Laboratory. Both of these persons have had extensive experience in the crime laboratory and both are expert in the use of electrophoretic methodology for blood analysis which utilizes gels as supporting media. In order to make all arrangements in an expeditious manner, it was decided to employ Mr. Wraxall and Mr. Stolorow as Beckman Consultants.

The consultants entered into an agreement with Beckman Instruments to be located at the WMRS, University of California, Berkeley, and to work on this program under the technical direction of Dr. Grunbaum. All data, experimental results, etc., resulting from their efforts of the Bloodstain Analysis Program were to be released through Dr. Grunbaum to Beckman prior to disclosure to others, including The Aerospace Corporation.

At the WMRS, these consultants were requested to set up and put in working order the electrophoretic equipment with which they were familiar. They applied their methodology in determinations of the variants under consideration for inclusion in the BAS and demonstrated convincingly that, in the hands of experts such as themselves, these methods can be useful. They provided the very best of the traditional methodology. This has been used as a basis of comparison with new methodology. They made some serious attempts to improve upon the old methodology, but achieved no significant improvements.

SUMMARY OF EFFORT

All technical support specified in the Statement of Work of the revised UC/Beckman subcontract was completed. The blood constituents specified in the Statement of Work and others were considered for inclusion as determiners in the BAS (Table 1). Likely candidates were selected from those constituents which demonstrated good resolution with fresh blood (Table 2). These constituents were subjected to aging tests, using 4-week-old blood. Analytical methodologies for the selected isoenzymes were optimized. A variety of substrates was considered and tested (Table 3).

At the conclusion of the Systems Definition phase of this program, the WMRS was prepared to suggest and defend a change in the Statement of Work that would eliminate the "three setup" requirement in favor of a vastly superior system utilizing simultaneous analyses. Feasibility Testing would have been carried out on schedule.

Another approach to the three setup system was proposed by the Beckman Consultant, Brian Wraxall. Since work on this approach had been carried out at the WMRS, we were in a good position to evaluate the probability of its success. It seemed highly unlikely that the analyses could be carried out on three setups. In any case, the simultaneous approach seemed far less likely to meet the other requirements of the Statement of Work than does the sequential approach.

Table 1

CONSTITUENTS CONSIDERED

FROM STATEMENT OF WORK	ADDITIONAL CONSTITUENTS CONSIDERED
HEMOGLOBIN, Hb	AMYLASE
HAPTOGLOBIN, Hp	URIDINE PHOSPHOKINASE
GROUP SPECIFIC COMPONENTS	GLYOXYLASE I
GLUTAMATE PYRUVATE TRANSAMINASE, GPT	GSR
RED CELL ACID PHOSPHATASE, EAP	BIS-ALBUMIN
PHOSPHOGLUCOMUTASE, PGM	TRANSFERRIN
ADENYLATE KINASE, AK	CERRULOPLASMIN
ADENOSINE DEAMINASE, ADA	ALPHA-1 ANTITRYPSIN
PSEUDOCHOLINESTERASE (E2), PCE (E2)	
6 PHOSPHOGLUCONATE DEHYDROGENASE, 6 PGD	
GLUCOSE 6 PHOSPHATE DEHYDROGENASE, G6PD	
ESTERASE D, EsD	
PEPTIDASE A, PEP-A	
CARBONIC ANHYDRASE	

Table 2

BLOOD CONSTITUENTS SELECTED AS DETERMINERS

Determiner	Discrimination Capability				
	Fresh Blood		30 Day Bloodstains		
	A	B			
EAP	.35	.43			.35
Gc	.45	.48			
PGM	.47	.48	.47	.47	.47
EsD	.69	.58	.69	.69	.69
Hp	.39	.39			
ADA	.82	.88	.82	.82	.82
AK	.82	.94	.82	.82	.82
GLO	.38	.38		.38	.38
Cumulative Discrimination Capability	(1 in 200)	(1 in 143)	(1 in 4.5)	(1 in 12)	(1 in 34)

A - Based on original estimate.

B - Based on data from this U.C. laboratory on about 12,000 blood specimens.
This work was supported by the Office of Criminal Justice Planning of the State of California and completed in September 1977.

Table 3

SUBSTRATES CONSIDERED

STARCH GEL
CELLULOSE ACETATE MEMBRANE
ACRYLAMIDE GEL
AGAROSE GEL
CELLO GEL
AGAR
SILICA GEL

Early in September, a "strong recommendation" was made by letter from Beckman ATO to concentrate research effort on System Development along the lines recommended by the Beckman consultant. This laboratory cooperated fully with the Beckman request until termination of the work in this laboratory on November 15.

Discrimination Probability

A technical requirement of the Statement of Work is that the electrophoretic analysis system developed be capable of achieving a degree of discrimination probability of one out of 200 randomly selected individuals, using as a sample a bloodstain aged for four weeks.

When this laboratory entered into an agreement to provide technical services as required to perform the tasks described in the subcontract, it was understood that it is theoretically possible to obtain a discrimination of 1 in 200 on bloodstains as old as 4 weeks. At the WMRS, methodology has been developed and is routinely used which permits discrimination of more than 1 in 250 on fresh blood. However, there are fundamental difficulties concerning the electrophoretic analysis of proteins and enzymes in aged blood or bloodstains. Physical and chemical changes occur in the character of these determiners which may affect their mobility and stability. If the changes are great, there is no possibility for a successful routine analysis. To date, we have no evidence that these changes can be reversed. IMPROVEMENTS AND CHANGES IN ELECTROPHORETIC METHODOLOGY WILL BE TO NO AVAIL IF THE DETERMINER ENZYME/PROTEIN HAS ALTERED THROUGH AGING AND DETERIORATION.

Proteins such as Gc and Hp may sometimes persist for a specified time in aged bloodstains stored under controlled laboratory conditions while at other times, under identical conditions, they will become undetectable.

What appears to be a great difference in the persistence of these enzymes among individuals may actually be due to innate differences in the concentration of these enzymes in their blood. Störiko (5) estimated that among individuals Gc may range from 25 to 50 mg per 100 ml serum. He estimated that Hp variations in fresh human blood may range from 10 to 220 mg in 100 ml serum.

In this laboratory we quantified the relative amounts of Gc and Hp in the fresh blood of several donors by a one-dimensional electro-immuno precipitation technique on cellulose acetate. In confirmation of Störiko's observations, wide variations among individuals were observed. It is obvious that on prolonged drying and degradation, some specimens will lose their entire Gc or Hp while others may still show substantial amounts and thus lend themselves for phenotyping. What may appear to be unreliability in an analytical method is, in fact, a function of concentration of the enzyme itself.

The systems PGM, EsD, ADA, and AK have proven to persist in 4 week old bloodstains kept under known laboratory conditions. These have a combined discrimination capability of 1 in 4.5 (Table 2). We were unable to determine Hp and Gc on 4 week old stains. GLO I may sometimes, but not always be determined in these aged stains. GLO I increases the discrimination capability to 1 in 12. If EAP is also determined, the cumulative discrimination capability is 1 in 34. For reasons stated below. EAP is not recommended for inclusion in the Bloodstain Analysis System.

The good frequency distribution of EAP makes it a very tempting candidate for inclusion in the Bloodstain Analysis System. The five common phenotypes, namely A, BA, B, CA, and CB, can now be readily and accurately determined using fresh blood (6). Bloodstains aged for 30 days on white cloth under

laboratory conditions has also been accurately typed. However, inherent characteristics of the EAP system give rise to the possibility of very serious errors in phenotyping on other than fresh blood, especially if the history of the sample is not known.

In this laboratory, an experiment was made to test the reliability and suggest the limitations of the EAP phenotyping in the crime laboratory. The experiment was designed and supervised by an expert criminalist from the Alameda County Sheriff's Department Crime Laboratory. Experimental stains and heat-degraded liquid samples were analyzed with great care by technicians having much experience with both cellulose acetate and starch gel methodology. The four readers, highly experienced with both methods of EAP phenotyping, had no knowledge of the phenotypes, the age of the samples, or the way in which they were prepared prior to analysis. The results showed that there can be a definite problem with the EAP phenotyping no matter which electrophoretic supporting medium is used. On cellulose acetate, the readers found several inconclusive and questionable readings on BA, B and CB bands. On starch gel, the readers "positively" misidentified several of the BA, B, and CB phenotypes.

Unlike other phenotypes, EAP depends not only on a pattern of relative distribution of bands, but also on the relative intensity of the bands. When blood is aged, the individual phenotypes tend to degrade at different rates, further exacerbating the true phenotype identification. In addition, EAP isoenzymes, on degradation, show so-called "storage bands" which are actually breakdown products of the protein molecule. These breakdown products are sometimes reversed by adding a reducing agent. However, questions always remain concerning the degree of restoration of the molecular structure. Because of the great number of variables affecting this enzyme system in vitro, phenotyping should not be attempted unless a complete history of the origin

and handling of a blood or bloodstain sample are known.

The selection of enzyme/protein determiners for inclusion in the BAS involved basic research into the stability and persistence of certain enzymes and proteins in dried bloodstains. It seemed apparent from the start that both The Aerospace Corporation and Beckman Instruments ATO understood the nature of this research and were prepared to make necessary changes in the Program Plan as new information developed.[3] Sensible people do not persist in a course of action when there is clear-cut evidence that the goals set cannot be achieved. It is good management to modify a statement of work when timely recognition of factors indicate that revision is necessary.

Methodology

Over the course of several months, before, during and after the System Definition phase of the BAS Project, excellent new methodology has been developed in this laboratory for the analysis of bloodstains for the selected protein/enzyme determiners. The 8 new methods are at least as sensitive and reliable as current methods, and they are considerably more simple, rapid, and easy to learn.

The methods for determination of PGM, EsD, EAP, AK, ADA and Gc involve use of cellulose acetate as a substrate. The new method[17] for the determination of Hp uses acrylamide nongradient gel as a substrate and reduces total analysis time for Hp to approximately 90 to 120 minutes.

A method[17] using an acrylamide gel substrate is also recommended for GLO-I.

When work on the BAS program began in January, this laboratory was

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[17] APPENDIX, Section A, See 3rd Interim Progress Report, 1-30 September 1977.

routinely doing enzyme/protein determinations of fresh blood, using cellulose acetate as a supporting medium.[18] Research was already underway to adapt some of this methodology for use with dried bloodstains. The successful adaptation of cellulose acetate methodology appeared attractive in terms of the increased rapidity of analysis and lower costs. Cellulose acetate methodology offers the added advantage of simplified documentation since the original patterns are permanently preserved. Accordingly, cellulose acetate was one of the several substrates tested in this laboratory. Several other supporting media were also tested (see Table 3).

There was no contractual requirement or agreement that blind tests or confirmation tests be run, and certainly no understanding that results of such tests would be used as the final basis for choice of methodology. These tests served as a useful challenge and research guide within the laboratory. They were certainly not designed carefully enough to be used in making critical decisions.

Each time a bloodstain was analyzed, we were in essence conducting a blind trial. However, in our efforts to assure that certain systems had reached a satisfactory level of development, formal blind trials were made. (Blind trials were made for EAP, EsD, and PGM.) The first of these trials was conducted in June and the results presented at our June program review meeting. Using the criteria that 90% of the stains must be identified correctly, none of the results of the first blind trial was satisfactory.[19] It was therefore decided to repeat the trials that were conducted in June. The results of these trials were reported in the Monthly Progress Report,

[18] These analyses are being done as part of a population survey financed by the Office of Criminal Justice Planning of the California Dept. of Justice. In the first year of this project, more than 100,000 analyses, including 10 genetic variants, were made on more than 13,500 blood samples.

[19] APPENDIX, Section C, Monthly Progress Report, July, 1977, p. 3.

July, 1977,[19'] from Beckman to Aerospace. It can be seen from Table I of that report that all three constituents were identified with a high degree of accuracy in four-week old stains.[20]

At the Program Review Meeting in August, Beckman presented EsD and PGM as equivalent on either starch gel or cellulose acetate. It reported starch gel as the preferred substrate for EAP. The results of the blind trials did not support this tentative decision concerning EAP, nor did prior and subsequent research results in this laboratory. In any case, as the Beckman Progress Report, July 1977, clearly indicated, no final decision on the choice of substrates had been made at that time.

The Beckman Monthly Progress Report covering work done in August gives results of Confirmation Tests in which all eight constituents were determined. Five of the methods recommended by this laboratory (for EsD, PGM, Gc, EAP and AK) on cellulose acetate were read with no errors. Another (ADA) was read with one error (less than 4% error). Our recommended methods were not used for GLO I or Hp. The starch gel and agarose gel methods which were included in these confirmation tests also demonstrated accuracy, even though there were several incorrect readings. However, they are not recommended by this laboratory because they are tedious and time-consuming.

[20] This Beckman report also contains the comment, "On cellulose acetate there were a total of 264 readings. Out of these there was a total of 4 called incorrectly and a total of 8 questioned. The 4 incorrect calls made on cellulose acetate were all by the same reader. Seven of the 8 questioned calls were also made by the same reader." It may also be added that this reader, a Beckman consultant, was the expert who had prepared the starch gel runs. On many occasions he has expressed a strong prejudice against the use of cellulose acetate and he had very little experience with the use of cellulose acetate.

System Development

As part of its survey task (1), Aerospace undertook a search of literature to identify promising methodology for use in crime laboratories and recommended approaches in developing a better blood identification methodology. The survey showed that several investigators had attempted to develop techniques for multisystem analysis on a single substrate (Goedde and Benkmann (7), Brinkmann and Dirk (8), Culliford (9), and Ritchey (10)). These efforts were all limited to starch gel, which requires a long analysis completion time. The Aerospace report suggested that it would be useful to extend such a technique to substrates which require shorter run times.

Wrede et al. (11) describe an attempt at determination of three enzyme polymorphisms on a single substrate. Polyacrylamide was used as the supporting medium in order, according to the authors, to avoid the well-known disadvantages of starch gel. However, this methodology involved suspending gels for 5 days in gel buffer, which was changed every day, then electrophoresing for 18 hours prior to specific enzyme staining. The method does not appear to offer a significant saving of time.

A relatively recent attempt at simultaneous analyses on starch gel is described by Neilson et al. (12). The authors' comments under "Results and Discussion" are most pertinent.

> "There are several precautions which should be stated when doing multiple enzyme systems. First, there are the considerations of pH and ionic strength which are not necessarily optimal for each enzyme studied. Second, in any multiple enzyme system, the rarer variant banding pattern of any one of the enzymes that overlaps into the developmental region of a second enzyme will not be visualized. Thus, multiple enzyme systems might best be used as a rapid screening test to observe basic enzyme variants."

For reasons of economy of space and equipment, it is desirable to have as few setups as possible in any bloodstain analysis system. The Aerospace Technical Requirements arbitrarily specified three "setups", defined as "no more than three electrophoretic substrate plates and no more than three cells". At the time this laboratory undertook to give technical support to this Project, this requirement seemed feasible.

After several months of basic research during the System Definition phase of the BAS, it became apparent that it would be difficult to achieve a 1 in 200 discrimination with four-week-old bloodstains even when these stains were preserved at carefully controlled laboratory conditions and analyzed under optimum conditions using the best available methods. These limitations are functions of the persistence of the proteins and enzymes themselves, as discussed above under the heading Discrimination Probability. It is the biochemical nature of these molecules that determines in great part how they will be affected through aging, and this, in turn, dictates optimal procedure for determination.

Strict adherence to the technical requirement for a three setup simultaneous system would involve compromises in methodology with resultant unreliability and ambiguity. These compromises would reduce discrimination probability. Analyst skill requirements would, if anything, be increased.

In this laboratory, the Aerospace Statement of Work and Technical Requirements were regarded as a reasonable point of departure. It was understood[3] that if, through basic research, evidence was presented that proved the technical requirements unachievable, the contractor (Beckman) would make appropriate proposals for revision of the Program Plan and submit these to the Aerospace Corporation for approval. We urged abandonment of a system of simultaneous analyses. We recommended that continued effort be

directed toward development of a system of sequential analyses where each of the determinations is made under optimal conditions, with no compromising of such variables as buffer concentration, pH, electrophoresis time, voltage, etc. for the sake of the letter rather than the spirit of the Statement of Work.

1. Sequential Analyses

The eight new methods recommended for inclusion in the BAS are at least as sensitive, reliable, and unambiguous as those selected by Beckman ATO. These new methods are superior in that they radically reduce both "hands on" and "analysis completion" times. They also require considerably less technical skill in preparation of samples.

The six methods using cellulose acetate produce an electrophoretogram which may be used as a permanent record, eliminating the need for photography, which requires time, expense, and skill.

A single Microzone cell (modified) or a Nanophor may be used as shown in Table 4 for sequential analyses. It is obvious that speed of analysis would be increased and more flexibility would be afforded if two or three cells were used.

When each determination is made separately, the protein/enzymes which are known to persist longest would be run first. A successful determination will indicate that the next analysis in the series should be made. An unsuccessful analysis could quickly be repeated. It may become apparent that because of physio-chemical changes in the sample due to aging and adverse conditions, the remaining analyses will be unsuccessful and so need not be done.

It is of course standard practice to repeat analyses to verify results. The proposed concurrent system is so fast and simple that all 8 analyses could be made and repeated in the elapsed time required for one run of the proposed Beckman system.

Table 4

SEQUENTIAL ANALYSES USING A SINGLE MICROZONE CELL (MODIFIED)

Order of Analysis	Substrate	Hands On	Elapsed Time
Sample Preparation	CAM	15 min.	15 min.
PGM	"	15 "	75 "
EsD	"	15 "	60 "
AK-ADA-EAP (simultaneous)	"	25 "	60 "
GLO-I	Acrylamide gel	30 "	150 "
Hp-Gc (simultaneous)	"	35 "	120 "
Total time		135 min. (2 hr. 15 min)	480 min. (6 h.)

2. Simultaneous Analyses

When it became apparent that Beckman ATO was not receptive to verbal arguments in favor of a request for a change in the Statement of Work, a written report[21] was submitted in early August. This report made specific recommendations in regard to methodology and equipment for a system of sequential analysis.

Rather than responding to the proposals from this laboratory, Beckman ATO sent a work plan[22] which assigned a schedule of specific tasks for grouping of constituents for simultaneous analyses.

The previously-mentioned September 6 letter[23] from Beckman ATO confirmed the administrative decision to continue with a system of simultaneous analyses.

Every effort was made to carry out Beckman ATO instructions until the November 15 termination date for technical support.

The Beckman consultant was given all laboratory facilities and technical assistance available under the BAS subcontract to work out simultaneous analyses on gel plates.

The selected determiners were grouped as follows:

I. PGM, EsD, GLO I

II. EAP, AK, ADA

III. Hp, Gc

When the support effort was terminated in this laboratory in mid-November, there was no successful system for simultaneous analyses for any of these groups. The Beckman Progress Report[24] for October gives the following

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[21] APPENDIX, Section A, Interim Progress Report on System Definition, 10 January to 31 July 1977.

[22] APPENDIX, Section B, See letter to B. Grunbaum, August 18, 1977.

[23] APPENDIX, Section B, See letter to B. Grunbaum, September 6, 1977.

[24] APPENDIX, Section C, 10th Monthly Progress Report, Nov. 10, 1977.

information:

The first group (PGM, ESD, GLO-I) is unsuccessful because of poor repeatability with GLO I.

Simultaneous analyses of group 2 (EAP, ADA, AK) has not been successful. Problems in determining ADA and AK are probably the result of necessary compromises in strength of reducing agents, buffer concentrations, and pH. Efforts to convert to 1% agarose/2% starch were made in an attempt to make the supporting media uniform for groups I and II. These efforts were not successful.

With the third group (Hp, Gc) the Beckman consultant reports that generally one will come out but not the other. The starch/agarose gel was also tried with this group, but did not work.

Research was also done on the grouping of Gc and Hp on cellulose acetate, using immunofixation techniques for localization and identification. However, it is the recommendation of this laboratory to use an established method of determination on Gc on cellulose acetate (13) and a newly developed method for determination of Hp on acrylamide gel.[25]

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[25] APPENDIX, Section A. Third Interim Progress Report, 1-30 September 1977.

DISCUSSION AND RECOMMENDATIONS

The proposed Beckman BAS

This system will not meet the discrimination probability requirements of the Statement of Work with any degree of reliability. This has to do with the physico-chemical modifications in dried blood rather than the methodology used.

It is unlikely that the system can be worked out using only three setups. When samples are determined separately, optimum conditions are marked out for pH, type of buffer, and buffer concentration. Analysis of multiple samples on a single substrate involves compromise which will affect results, especially when aged samples are used. The slightest modification in conditions of electrophoresis will change mobility in such a way that patterns may overlap. With marginal samples, decomposition products may obstruct clear patterns, and if chemicals are added to help one system, they may adversely affect another.

The problems described above in relation to a three setup simultaneous analyses is not dependent upon choice of methodology. However, for other reasons, the choice of methodology is a matter of overriding importance. The Aerospace report comments as follows on the efforts of LEAA to promote the Culliford methodology:

> "Although this activity has generated considerable interest in past years, very limited use of serological results is currently made within the criminal justice system in the U.S. It is this lack of response to past efforts by LEAA which has led to The Aerospace Corporation program to improve analysis methodology to that level of performance required to ensure greater utilization of blood individualization in the U.S."[26]

[26] The Aerospace Corporation SURVEY AND ASSESSMENT BLOOD AND BLOODSTAIN ANALYSIS PROGRAM, Vol. I, p. 6.

The Aerospace Statement of Work advises in regard to the Culliford approach, "This procedure is to be considered as a baseline only, to be improved upon or replaced by the development effort required by this Statement of Work".[27]

It is unfortunate that after many months of work and considerable expenditure of public research funds, Beckman ATO proposes to offer the Aerospace Corporation a system of slightly modified Culliford methods. Because of the complexities imposed by the Technical Requirements[28], this methodology is now more complicated, more difficult to learn, more dependent on analyst skill, and less accurate than ever. There is no possibility that it will ever be used routinely in U.S. crime laboratories.

Claims that this methodology now requires less "hands on" time and less time for analysis completion appear to be considerably exaggerated. However any claim concerning time saved is irrelevant since the system itself is not practical for routine use.

Since government research funds have been expended, and since the facilities of a public university are involved, it is proper that any justification for the major administrative decision by Beckman ATO be fully understood. The 10th Monthly Progress Report gives a less than factual rationale for this choice:

> ".... However, the data from tests on this program show that the time requirements for technician effort is about the same with either substrate. Further, and most important, are the results which show that group analyses are most likely to be made successfully with the gel substrate. Therefore, based on the test results to date, it was decided to concentrate development effort on the gel plate methodology. This approach has shown the greatest potential to meet the requirements of the statement of work."[29]

[27] The Aerospace Corporation, STATEMENT OF WORK FOR A BLOODSTAIN ANALYSIS SYSTEM, p. 3.

[28] Statement of Work (Aerospace), Annex C.

[29] APPENDIX, Section C, Tenth Monthly Progress Report, Nov. 10, 1977, p. 1.

In fact, there were no tests performed in this laboratory to determine time requirements for technician effort for either substrate. There were no tests performed in this laboratory that show that group analyses are more likely to be made successfully with the gel substrate. If such tests had been made, they certainly would have been recorded and reported. No such test results are given in the Monthly Progress Reports from Beckman ATO or in the Viewgraph presentation made by the Beckman Program Manager at the Program review Meeting on October 3, 1977.

The Beckman ATO decision was not based on test results. The BAS project administrators were faced with a dilemma. On the one hand, the Project Director at WMRS urged revision in the Statement of Work to permit a sequential system of analysis, arguing that the proposed three setup system for simultaneous analyses was probably unachievable and most certainly impractical. On the other hand, the Beckman consultant consistently made extremely optimistic though unsubstantiated claims that his approach, utilizing an agarose/starch gel methodology, held great promise of meeting the requirement of the Statement of Work. Exercising its unquestioned prerogative, Beckman BAS Program management made an arbitrary choice.

Parallel Placement of Samples for Simultaneous Analysis

It was suggested that parallel rather than perpendicular placement of samples on the supporting gel plates will facilitate simultaneous analyses by preventing overlapping. This innovation should avoid some of the difficulties that arise because of inconsistency in the gels from batch to batch (14). This is particularly true with agarose gel. Imperceptible varying amounts of trace contaminants may radically alter electrophoretic patterns as a result of electroendosmosis, which is difficult to control.

There is always the possibility that overlap and too-close margins will result in loss of bands when phenotype patterns are determined in the perpendicular fashion. Thus it is likely for laboratories using agarose gel from different sources to obtain conflicting results on the analysis of the same bloodstain specimen.

In the parallel fashion the bloodstain is placed in the gel with appropriate standards on either side. Since the proposed setup using the NANOPHOR Electrophoresis System can accommodate 12 samples, it would thus be possible to arrange for the simultaneous analyses of any group of three determiners that can use a common pH and buffer. At the end of electrophoresis, the overlays with appropriate substrates would be placed parallel to the electrophoretic axis of each variant and there is no possibility of loss of information due to overlapping or failure to cover any of the bands, regardless of the quality and makeup of the gel.

Should this suggestion be adapted, it would facilitate a technical improvement in the proposed Beckman ATO system. However, the basic objections to simultaneous analyses would still remain.

Future Research

By the time the final report of the BAS is completed by Beckman Instruments, Inc. and The Aerospace Corporation, the arguments concerning the use of starch gel vs. cellulose acetate may be irrelevant. It is now known that separation of proteins in a linear pH gradiant produces an unusually high degree of resolution by separating proteins according to their isoelectric point. Electrofocusing on polyacrylamide gel not only results in many electrophoretic zones, but each zone is also highly concentrated as an inherent feature of the technique. Thus, for instance, in the EAP system,

Kaczmarek (15), using a highly purified enzyme, showed 21 isoenzymes for a given phenotype, while conventional electrophoresis shows only two or three (so-called primary, secondary, tertiary bands). Using isoelectrofocusing for typing the common PGM variants, Bark et al. (16) demonstrated 10 new variants. Thus they increased the discrimination probability of PGM from about 1 in 2 to 1 in 4 individuals. This is of tremendous significance when examining bloodstain evidence. Grunbaum (17) in 1975, working with dried bloodstains found about 60 bands (zones) after isoelectric focusing. On the basis of this finding, a system was proposed which would make it possible to determine 5 to 10 genetic systems simultaneously. Subsequently Burdett and Whitehead (18) reported simultaneous separations of several polymorphic systems.

It should be noted that the Beckman proposal did recommend the evaluation of the technique of isoelectric focusing to determine its potential for methodologic simplification. It is unfortunate that Aerospace chose to delete all studies related to the use of isoelectric focusing for electrophoresis from the BAS Statement of Work.

The Beckman Proposal also included an approach for the definition of "markers" which would indicate whether components are seriously degraded. Aerospace requested deletion of all enzyme marker studies related to determination of age of stain for ascertaining whether the stain can be easily analyzed by electrophoresis. Independent of the BAS, this laboratory has carried out the marker system studies, with useful results. Using the highly sensitive and specific electro-immuno-precipitation method, it is possible to assess the relative amounts of Hp and Gc remaining in a bloodstain. This test is carried out on cellulose acetate using the unmodified Beckman Microzone cell. If no

Hp or Gc shows up in these tests, phenotyping by electrophoresis for these variants is probably of no use. If Hp and Gc are present to any extent, the chances are that the stain is relatively fresh or well preserved and warrants the complete analysis for all variants.

Conclusion (Options for the BAS)

At some time during the system development phase of the BAS project there appeared to be an acceptance on the part of Beckman ATO that the Technical Requirement for "three setups" was of paramount importance. The Project Director at WMRS was convinced that this requirement was important only if it increased capability to meet those other technical requirements which would determine the system's ultimate usefulness and acceptability in U.S. crime laboratories. Consequently, two approaches to system development evolved. It was Beckman ATO's prerogative to choose one over another, and it chose to continue development of a three-setup system. The alternate system, one of sequential analyses, required little additional development. With continued BAS project support, including appropriate equipment supplied by Beckman ATO, this would most certainly have been ready for feasibility testing by the beginning of December 1977. It would have perhaps been worth the investment of minimal additional financial support to have two systems available for comparison in feasibility tests and subsequently for crime laboratory testing and evaluation.

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APPENDIX

SECTION A

TO: Advanced Technology Operation
Beckman Instruments Inc.
Anaheim, California
Attn: Mr. John Walsh

INTERIM PROGRESS REPORT ON SYSTEM DEFINITION

10 January to 31 July 1977

Subcontract: Z-847905-6

Subcontractor: University of California
Berkeley, California 94720

Submitted by: Benjamin W. Grunbaum
Principal Investigator

RATIONALE FOR SELECTION OF METHODOLOGY

This project is justified on the basis that the traditional methods of electrophoretic analysis for determination of genetic variants in blood, though known for years, are simply not being used in American crime laboratories. Newspaper accounts recently publicized results of a federally-funded three-year project to test the capabilities of 240 crime laboratories operated by federal, state, and local law enforcement agencies.[1] The release reported a wide range of error in response to tests including bloodstains. Only 40% of the laboratories tested gave correct answers on a bloodstain comparison test. Of 124 laboratories performing the test, only 49 correctly reported that the two bloodstain samples did not come from the same person. The others made incomplete or inconclusive reports because they lacked the ability to perform the more sophisticated tests or made mistakes in the tests.

An earlier survey was made for LEAA by the Aerospace Corporation "to assess the state of the art of bloodstain analysis in the U.S. Crime laboratories and to establish the specific needs of forensic serologists for improvements in technology."[2] Special efforts were made to include those laboratories that were reported by others to be performing blood analyses; thus the survey was more indicative of the extent of blood evidence use in larger and better-staffed laboratories. The survey revealed that even these facilities were making very limited use of available technology.

One of the reasons offered by Aerospace for the limited use of bloodstain clues was "a lack of simple, rapid, and inexpensive analytical methods for

[1] United States Department of Justice, Law Enforcement Assistance Administration News Release, Washington, D.C., 8 April 1977. This study was conducted by the Forensic Sciences Foundation, for LEAA

[2] Survey and Assessment. Blood and Bloodstain Analysis Program. Volume I, Technical Discussion. El Segundo, California. The Aerospace Corporation, April 1974, p. 17.

the detection of the genetically derived blood constituents which will positively identify an individual. Available techniques are, for the most part, time consuming and often yield ambiguous results."[3]

It would be self-defeating, in the face of such a clear and concise analysis of the problem by Aerospace, for this project to end up with a System which incorporates only, or mostly, the old methodology which the U.S. crime laboratories have been unable to utilize.

The minutes of the Program Review (June 20, 1977) states, and the subcontractor concurs, that the only acceptable end product of this research is the development of a system(s) which will benefit the crime laboratories. It has been our objective to develop new methods for bloodstain analysis with the expectation that they will be reliable, accurate, economical, rapid, easy to learn, and easy to perform in the crime laboratory.

The subcontractor is not concerned with any so-called controversy between starch gel and cellulose acetate, but only with the development of reliable and reproducible methodology in all systems for the purpose of upgrading forensic serology and aiding in the administration of justice. Accordingly, we are recommending for inclusion in the Blood Analysis System some methods which use gel and some which use cellulose acetate.

I suggest that we do not commit ourselves to any system of methodology which excludes further development and expansion. The search for improved technology must continue even after the completion of this project. Different approaches are already under consideration (Grunbaum, B. W., Some New Approaches to the Individualization of Fresh and Dried Bloodstains. J. Forensic Sciences, 21: 488-497, 1976), and will most likely prove superior to conventional electrophoresis using either starch gel or cellulose acetate as a supporting

[3] Survey and Assessment. Blood and Bloodstain Analysis Program. Volume I, Technical Discussion. El Segundo, California. The Aerospace Corporation, April 1974, p. 19.

medium. The crime laboratory must be supported on a continuing basis with research designed to improve the state of the art.

METHODOLOGY

In developing a Bloodstain Analysis System it was imperative to have at hand primary parameters for comparison. Such basic parameters must of necessity be established on fresh blood. In this laboratory many thousands of fresh blood specimens have been analyzed prior to and during this contract. The bloods are obtained from various blood banks; the samples are sterile and of transfusion quality. Ten different genetic variants are routinely phenotyped. Seven of these systems belong in the group of eight selected for a discrimination capability of 1:200 in the Bloodstain Analysis System Project.

The accompanying chart lists the eight variants which have been studied for determination in the proposed Bloodstain Analysis System. Seven of these are recommended without reservation. The chart (Table 1) also gives the recommended support medium for the electrophoretic determination of each of these variants.

The variants were selected on the basis of their reliability, persistence, and discrimination capability. The cumulative discrimination capability of these eight variants is 1 in 200.

Man Hours

The work for a determination using cellulose acetate includes preparation of the stain extract, setting up the standards, preparing the electrophoretic apparatus, application of the sample, phenotype development, and reading and recording the results. The work for the determinations involving starch gel include, in addition, preparation of the starch gel plate. When gel is used a stain solution is not required. Instead, a bloodstained fiber is prepared and imbedded into the gel. The cumulative man hours required for these eight

methods is less than five hours even if each determination is done on a different stain. When all determinations are made on a single stain, one stain solution can be prepared for all six determinations on cellulose acetate, thus reducing man-hour time by about 75 minutes.

The length of time given on the chart must be approximate because it will vary with the capability and expertise of the technician.

Elapsed Time

The two methods using a gel as a supporting medium require approximately 24 hours for analysis. The six methods using cellulose acetate can all be done with comfort in a single four-hour period.

EAP

A good frequency distribution makes EAP a useful discriminator. The recommended method of determination, using cellulose acetate, has been repeatedly tested in conformity with the technical requirements of this project and the phenotypes were identified without ambiguity.

However, now that I have studied EAP determinations using both cellulose acetate and starch gel as supporting media, I suggest that we consider very carefully before we include this variant in the Bloodstain Analysis System. Following are some of the reasons I question its suitability for dried bloodstain analysis in the crime laboratory.

1) Unlike other phenotypes, EAP depends not only on a pattern of relative distribution of bands, but also on the relative intensity of certain bands. For instance, even when blood is fresh slight differences in the intensity of the C band may confuse it with either a homozygous phenotype C or a heterozygous phenotype CB, even though both isoenzymes are present. When blood is aged, the individual phenotypes tend to degrade at different rates, further exacerbating the true phenotype identification. In addition,

the EAP isoenzymes, on degradation, show additional so-called "storage bands" which are actually breakdown products of the protein molecule. This is usually corrected by adding a reducing agent. However, the question always remains:- Has the molecule structure truly been restored to its native state? If it has not, then how can one be sure about its relative electrophoretic mobility and its eventual reactivity with a specific substrate?

2) In the phenotyping of many thousands of fresh blood samples for EAP, there has been no difficulty in determining the five common phenotypes (A,B,BA,CA,CB) and many rare variants. When either cellulose acetate or starch gel is used, resolution is the same for all phenotypes except A.

It is presently agreed that the homozygous A phenotype consists of 2 bands; the major one, A, has a mobility faster than the B band, while the minor one, A' (A prime), has a mobility slower than the B band. On cellulose acetate, the homozygous A clearly shows two bands. However, in the phenotype BA, the A' band is masked by the B band. This does not interfere with accurate phenotyping. On starch gel, using fresh blood, the A' band is clearly visible but it neither increases nor decreases accuracy in identifying the phenotype.

In 30-day old bloodstains extracted from cloth, the major A band was clearly visible after electrophoresis on cellulose acetate. Thus again the accurate phenotyping was independent of the A' band.

The A isoenzyme degrades faster than the B or C isoenzymes. It is possible, therefore, to call a true BA phenotype a B. However, when starch gel is used, the A' may still remain after the A has disappeared. Although there has been some suggestion that a phenotype can be identified as BA when only a B band and an A' band is visible, there is certainly room to question the reliability of such a determination. It is possible, for instance, that

the apparent A' is really a storage band. Conversely, if A' is not present, it cannot be concluded that the phenotype is a B; the A' may simply have disappeared along with the A band as the blood aged. Certainly such determinations could be challenged in a court of law.

There is, of course, a solution to avoid this problem, regardless of whether cellulose acetate or starch gel is used. By comparison with appropriate aged standards such uncertainties could be resolved. Development of such standards is beyond the scope of this project.

PGM

Methodology using cellulose acetate in the phenotyping of PGM in bloodstains has been worked out in a California crime laboratory (Zajac, P. L. and A. E. Sprague, Typing of Phosphoglucomutase (PGM) Variants in Dried Bloodstains by the Grunbaum Method of Cellulose Acetate Electrophoresis. J. Forens. Sci. Soc., 15: 69-74, 1975). Results of this PGM phenotyping have been presented in courts-of-law as evidence numerous times. This method has been tested for this project with satisfactory results.

EsD

Methodology for determination of this variant in fresh blood was adapted for use with dried bloodstains in this laboratory. The present methodology, using cellulose acetate membrane, is quite satisfactory for unambiguous phenotyping. Thirty-day old specimens extracted from cloth show considerable degradation. However, by varying the strength of the membrane buffer, it is possible to concentrate the individual electrophoretically separated isoenzymes. This yields higher intensity per unit band width, better band separation, consequent superior resolution, and unambiguous phenotyping. Unlike starch gel determination of EsD, the CAM does not show any other esterases which tend to obscure proper phenotyping.

AK, ADA, 6PGD and Hb

The above systems have low discrimination indices but are simple to determine in fresh blood on cellulose acetate. Furthermore they separate in such a manner that all four systems can be determined simultaneously from a single sample. This methodology has been adapted for phenotyping for AK on cellulose acetate using 30-day old bloodstains from cloth. Adaptation of methodology for determination of the other three systems is progressing well, but only ADA is presently included in the group of 8 systems.

Hp

Haptoglobin phenotyping has been successfully done on 30-day old bloodstains using acrylamide gel as the supporting media. It is agreed that this is the best method presently available. O-dianisidine rather than o-tolidine will be used as the stain because it results in a permanent phenotype pattern. We have found that "cleansing" the bloodstains with chloroform improved the resolution considerably.

Work is underway in this laboratory to develop a much improved and simplified procedure for Hp, but this is not being done as part of the Bloodstain Analysis System Project.

Gc

Determination on cellulose acetate (Grunbaum, B. W. and P. L. Zajac, Rapid Phenotyping of Group Specific Component by Immunofixation on Cellulose Acetate. J. Forensic Sciences, July 1977) has proven the fastest, simplest, and most economical means for phenotyping Gc variants. The adaptation to bloodstains has been worked out. Cellulose acetate electrophoretograms show that as a stain ages degradation products accumulate increasingly in the form of a diffuse anodic band. Apparently the breakdown products of the Gc molecules still have the capacity for specific interaction with antiserum. Fortunately

this does not interfere with phenotyping. However, as the degradation products increase, the remaining intact Gc molecules yield fainter bands and phenotyping becomes marginal. As a result, some determinations on 30-day old bloodstains were negative. However, since stains as old as two months have been successfully phenotyped, it must be assumed that concentration levels of Gc vary between individuals.

GLO I

A new genetic marker, red cell glyoxalase I, was recently described (J. Kömpf, S. Bissbort, S. Gussmann and H. Ritter. Polymorphism of Red Cell Glyoxalase I (E.C.: 4.4.1.5). A New Genetic Marker in Man. Humangenetik 27: 141-143, 1975). It has a discrimination probability of 0.38. Attempts to resolve the heterozygous GLO I 2-1 into 3 distinct bands on cellulose acetate have been unsuccessful to date. Because of the scheduling limitations of the Bloodstain Analysis Project, I suggest the starch gel method be accepted. On this electrophoretic medium GLO I appears to separate into the 3 common phenotypes.

If time permits, additional trials to separate GLO I on cellulose acetate will be made.

COMMENTS ON THE NEXT PHASE OF WORK

The next task of the subcontractor will be to integrate the methods of choice into a system that will be convenient and easy to use, accurate, reliable, efficient, and economical.

Rather than narrowly following the letter of the Technical Requirements, we must strive to produce the best system possible at this time to meet the need of all crime laboratories, especially those who, for a variety of reasons, have been unable to utilize existing methodology in the development of bloodstain evidence.

The Aerospace Technical Requirement proposes a system "using no more than three electrophoretic set-ups". When this requirement was introduced, it was perhaps supposed that a single electrophoretic supporting medium could be found and that a common buffer could be used to resolve at least several genetic variants, either from the same sample or from several samples on a single supporting medium.

It is now clear that three media, acrylamide gel, starch gel, and cellulose acetate, are needed. I suggest that our primary objective be to set up a system where each of the analyses is done under optimal conditions, with no compromising of such variables as buffer concentration, pH, electrophoresis time, voltage, etc. for the sake of the letter of the Statement of Work.

The Nanophore, developed in this laboratory with NASA funding, offers an unparalleled flexibility, unlike any commercial instrument, for a variety of electrophoretic and associated techniques. It is suited for electrophoresis using all known supporting media. The apparatus is compact; it features (1) a temperature control plate, (2) two electrode systems, one for conventional electrophoresis, the other for electrofocusing, (3) a 10-sample automatic applicator, (4) a sample-indexing system, and (5) a 60-sample holding plate. Two or more Nanophores can be integrated to form a single "set-up" which uses a single power supply. An apparatus of this kind should serve well as standard equipment in the crime laboratory.

TABLE 1

	Hp	Gc	EAP	GPM	AK	ADA	EsD	GLO I
Supporting Medium	Acryl. Gel	CAM	CAM	CAM	CAM	CAM	CAM	Starch Gel
Discrimination Probability	0.39	0.45	0.35	0.47	0.82	0.82	0.69	0.38
Man Hours Analysis	55 min	35 min	35 min	40 min	40 min	40 min	40 min	45 min
Elapsed Time Per Analysis	16 hrs	1.0 hrs	1.5 hrs	2.0 hrs	1.5 hrs	1.5 hrs	2.0 hrs	
Yields Unambiguous Results	U	U	U?	U	U	?	U	?

TO: Advanced Technology Operation
Beckman Instruments Inc.
Anaheim, California
Attn: Mr. John Walsh

SECOND INTERIM PROGRESS REPORT ON SYSTEM DEFINITION

1-31 August 1977

Subcontract: Z-847905-G

Subcontractor: University of California
Berkeley, California 94720

Submitted by: Benjamin W. Grunbaum
Principal Investigator

During the month of August research has continued in two main directions. The first was to further improve methodology for individual constituents using cellulose acetate, especially with regard to pattern resolution and recognition of phenotypes. The other area of research involved the combination of systems for simultaneous determination of several variants.

In addition, work was resumed in the area of electro-immuno-precipitation (EIP) methodology on cellulose acetate for the following purposes:

a) Identification of presence and/or quantitative determination of specific proteins such as the Group Specific Component, Haptoglobin, Transferrin, and others by one-dimensional EIP.

b) Phenotyping of Gc and Hp by two-dimensional EIP.

Comments on the individual variants

PGM. At this time one of the drawbacks in the use of cellulose acetate in bloodstain analysis is the need for multiple application of sample extract. This tends to start the electrophoretic separation with an origin too wide for good resolution. Also, because the separated bands are diffuse, that is low concentration of PGM isoenzymes per unit area, they require longer development time over the substrate for visualization and identification. Even though the resulting patterns were recognizable they were diffuse. Also because of the length of development time, the background staining with formazan further reduced the contrast.

The above difficulty was remedied by reducing the number of specimen applications (bloodstain extract) to half or less, usually two applications instead of 4 or more. Also the development time was reduced to where the standards were clearly visible and the bloodstain extracts barely visible. This procedure had the effect of producing sharper zones and better contrast. Thus, the separation of the PGM b and c band was actually achieved. Since visualization of isoenzyme patterns on cellulose acetate is best done by viewing with transmitted light, the above procedure was very effective. Of course the extent of a visible pattern depends mainly on the concentration of soluble PGM in the extract. However since the concentration of PGM in a bloodstain extract is not known a priori, it will be necessary when dealing with a bloodstain to apply increasing quantities to a membrane with one single application. This should result in at least one pattern of sufficient quality for unambiguous identification.

Trials were also made to intensify individual PGM bands by increasing the buffer concentration (from the present 1:20 soak) in the membrane. Although the zones were indeed sharper, they were too close to each other for adequate separation.

The most urgent need at present is the manufacture and use of sample applicator tips of varying quantities to avoid the need for multiple applications.

EsD. Unambiguous identification of phenotypes from 30-day old bloodstains on cloth was obtained on all specimens tested. At a cellulose acetate membrane buffer concentration of 1:7, the bands are very sharp. However I decided to use a 1:10 dilution of tank buffer for the membrane to allow more flexibility relative to electrophoresis time. At the higher concentration the amperage was more likely to rise sharply, causing thermal convection. The somewhat lower buffer concentration (1:10) permitted longer electrophoresis time if necessary. The pattern characteristics were not affected.

Pattern definition is also a function of sample size for EsD and any other system (see PGM above). The general rule is that the smaller the sample size the better the resolution. This principle worked favorably for EsD.

AK. This is a very persistent enzyme system and there is no problem in phenotyping 30-day old bloodstains on cellulose acetate.

EAP. The phenotyping of this system continued to yield excellent results using 30-day old bloodstains from cloth, which were prepared in this laboratory. Glycerine (15%) is added to all substrates. The glycerine addition has a dramatic effect on the speed of pattern development and pattern preservation within the cellulose acetate membrane for future repeated viewing. Since the glycerine impedes diffusion, the individual bands remain sharp. Since the function of glycerine is to remove the end product (phosphate groups) from the enzymatic action of EAP, the resultant is a corresponding increase of the fluorescent compound 4-methyl umbelliferone. This in turn heightens the intensity of the EAP patterns relative to a non-glycerine containing substrate. Note that the longer the time requirement for enzyme-substrate interaction, the more diffusion will occur. In a paper submitted for publication I explained the difference in the physical effect that glycerine has when using starch gel or cellulose acetate. With the present starch gel methodology, the glycerine effect may be ineffective or undesirable.

Extensive experimentation during this period was done to compare the effect of glycerine on EAP cellulose acetate electrophoretograms versus no glycerine.

Five phenotypes were observed, namely: AA, AB, BB, CA and CB. The CC pattern was not available for study. Using fresh blood, the glycerine inclusion allowed phenotyping of EAP after 5 min. The zones were tight and the pattern bright. It required about 15-20 minutes for pattern visualization when no glycerine was used. The zones were wider and "dull" in comparison with those on glycerine. In some CB phenotypes the C and B bands were of equal width when no glycerine was used. With glycerine the C band appeared imperceptibly narrower relative to the B band but of the same intensity. There was never any more or less confusion in calling a phenotype CB whether glycerine was employed or withheld. The equal widths of C and B without glycerine may be either because of diffusion or individual differences. Many more CB types need to be examined.

EAP phenotyping on cellulose acetate is one of the most successful determinations we have developed, especially because of the inclusion of glycerine. In dried blood analysis for EAP the element of glycerine inclusion is of paramount importance because of the expected degradation of enzyme systems, and EAP in particular.

ADA. Using freshly prepared substrates, no difficulty was encountered in phenotyping for ADA on cellulose acetate.

Gc. Not all 30-day old bloodstains yield recognizable patterns for identification when analyzed by immunofixation on cellulose acetate. It appears that the persistence of this protein in bloodstains (as prepared in this laboratory) varies considerably among individuals. However, it is more likely that the apparent differences we note are due to concentration variations among individuals in their blood rather than persistence.

I tested this hypothesis by quantifying the relative amounts of Gc among individuals by a one-dimensional electro-immuno-precipitation technique (EIP) on cellulose acetate. Wide variations among individuals were observed. (The EIP on cellulose acetate is fast (60-80 min) and can be retained as a permanent record.) Störiko[1] estimated the Gc between individual humans to range from 25 to 50 mg per 100 ml serum. It is thus obvious that on prolonged drying and degradation some specimens will lose their entire Gc content, while others may show substantial amounts and thus lend themselves for phenotyping.

Hp. It appears possible now to phenotype bloodstains using a homogeneous 6% acrylamide gel slab, using the Beckman Microzone Accessory. Repeated analyses will have to be made to confirm it. But as with the Gc system, some bloodstains do not produce a visible identifiable pattern. Again we quantified fresh specimens of human blood by EIP for Hp on cellulose acetate. We found them to vary even more than for Gc. Störiko estimated the Hp variations in humans to range from 10 to 220 mg in 100 ml serum. Again, it is thus apparent that some bloodstains will lose their Hp on degradation while others will exhibit strong Hp phenotypes. One must take into account the obvious fact that humans exhibit a natural and normal individual variation in just about every constituent in the blood. If a constituent is detected by a given method in one individual and not in another, it is not the method at fault. The electrophoresis time for Hp from bloodstains has been reduced to 90 min, which is the same as always used for fresh blood. The next simplification in Hp phenotyping will be use of one millimeter thick acrylamide gel slabs and cooled on one side only.

Phenotyping of Gc and Hp by Electro-Immuno-Precipitation (EIP) on Cellulose Acetate

We demonstrated that cellulose acetate membranes are excellent substrates for EIP. The one-dimensional process for "rocket" formation could be accomplished in about 60-80 min using the original Microzone cell and cooling was not required. For the two-dimensional EIP which is necessary for phenotyping, the original Microzone cell could also be used, but not with the same ease as the Nanophore which was designed for such use. In any case, the Gc by EIP exhibited a single rocket for type 2, a double rocket for type 1, and a triple rocket for type 1-2. Furthermore, the lateral displacement of the individual polymorphs from the point of origin were equivalent to the mobilities obtained by immunofixation. The total time required was 75 min and 3 specimens were run on the same membrane using the Nanophore. No cooling was required. On the Microzone system only one

[1] K. Störiko, in Blut, Vol. XVI, pp. 200-208, 1968. J. F. Lehman, Publisher, Munich.

specimen can be phenotyped by EIP.

It appears that Hp can similarly be typed, but more experiments are needed with different anti-human haptoglobin antisera.

Since the Gc and Hp have considerably different mobilities, it might be possible to combine the antisera, then phenotype both simultaneously.

Additional attempts were made to phenotype Hp by immunofixation. An evaluation should be available during the month of September.

Simultaneous systems determination using a single "setup"

It appears that the 1:200 discrimination capability will be obtained using the methodologies under investigation. It also appears that with "ideal" 30-day-old bloodstains on cloth, such as prepared in this laboratory and analyzed by expert technicians, certain simultaneous determinations can be made. Combinations such as glyoxylase, PGM, and EsD can be done on starch, and agarose gel containing starch. Gc and Hp can be done simultaneously by two-dimensional EIP on agarose gel. On cellulose acetate, 4 systems can be determined simultaneously when run in parallel. These variants are PGM, EsD, AK, and ADA.

However, the patterns obtained on all the above attempts are not nearly as clear and readable as when these determinations are made separately.

At this time we still need three different types of substrates and three electrophoresis instruments of different design and manufacture.

Our choice now is whether we put together a 3-setup system for simultaneous determination that will be regarded merely as a "gimmick" by the forensic laboratories, and which will never be put to practical use, or whether we design the most efficient, reliable, economical, and practical system of methodologies that is possible with our present knowledge. It is my feeling that forensic laboratories are more likely to adopt a system including concurrent methods that are quick and efficient and yield excellent results, than a simultaneous system with marginal results.

We must remember that we are preparing this system for technicians who must have specialized skills in the analyses of many kinds of physical evidence, and cannot devote themselves solely to bloodstain analysis. We cannot expect them to have the time to develop the expertise to perform highly complicated procedures of bloodstain analysis or to interpret marginal results. Therefore, we will serve the forensic laboratory best by giving them the best possible methods, without sacrifice to some arbitrary criteria.

In my interim report for the period 10 January to 31 July 1977, I expressed my doubt that the needs of the forensic laboratory could best be met, in the time and with the money available for this project, by adhering rigidly to the requirement that three "setups" be used for the simultaneous determination of all constituents, for a discrimination capability of 1:200.

I suggest that we consider again my proposal in the last report for a NANOPHOR setup, or that we adopt the modified Microzone system as it is used in my laboratory.

Relative merits of supporting media

A. Fibers versus extracts

During the last seven months we benefited from the best technical expertise in the use of starch gel for bloodstain phenotyping. During this period the procedures using starch gel have been simplified by shortening the electrophoresis time and by using smaller gel plates. However, in principle the method did not change much. For each electrophoresis run a fresh starch gel has to be poured and the gel cured and set carefully. A stained thread has to be carefully inserted into the gel without causing too much damage. This requires considerable skill as up to eight or more individual threads must be inserted in an absolute straight line.

The use of single thread poses another question. Just how representative is a single thread when teased out of a stain? Whole blood is not a homogeneous solution. About 40% consists of red blood cells. When blood is drawn into a test tube or capillary, these cells settle out very rapidly. When blood is deposited onto cloth or any other absorbent material, the red blood cells may become quickly enmeshed in the fibers, while the soluble portion, the 60% serum, may continue to diffuse outside the visible periphery of the stain. Fibers removed from such a stain may differ in their blood content and thus reflect variations in intensity for given constituents.

An extract of stain is therefore more representative of the whole blood. On repetitive examinations, it should yield identical results. In the cellulose acetate procedures only bloodstain extracts are used.

B. Time of analysis

When dealing with bloodstains, there is no way to predict a priori the survival of active proteins and enzymes. When electrophoresis must be repeated because of uncertainties in phenotyping, it would usually take 36-48 hours for starch gel and not more than 4 hours for cellulose acetate. This should be of considerable advantage.

C. Documentation

There is just no way a starch gel electrophoretogram can be preserved as an original, so a photograph is required. Polaroid photos are fast and generally of low quality. High quality photographs require considerable development time. But the gel patterns do not last long enough to permit the taking of a second set of pictures if the first set of pictures does not turn out well enough. In reality, no photograph is ever truly as good as the original.

On cellulose acetate the original patterns are permanently preserved. There is no requirement on the criminalist to be either an expert photographer or to apologize for bad pictures.

Very soon a firm decision will have to be made concerning the choice of substrate for the determination of each of the constituents.

My recommendations will be based on the above considerations.

Comments

Attached to this report is a copy of a table in an article by B. W. Grunbaum which will soon appear in Mikrochimica Acta. In this table 20 different polymorphic protein systems are shown to be determined on cellulose acetate. The trend towards cellulose acetate and away from gels is also demonstrated by the increasing number of papers using the ready membrane (see paper by Kearney and Stombaugh of the FBI in the July issue of J. Forensic Sciences).

Attached is also a copy of the program of the meeting of the Southern Association of Forensic Scientists. The meeting was held in Orlando, Florida on 26, 26 and 27 August 1977.

The "workshop" again convinced me of the veracity of Aerospace conclusions that the criminalistics community in this country needs a different approach to bloodstain analysis, other than that offered by the outdated starch gel techniques. I had the identical impression when I visited the forensic laboratories in Poland (during June) as an observer for the National Academy of Sciences.

Table I. Polymorphic Enzymes and Proteins Determined by Electrophoresis With the Microzone System[a]

Enzyme/Protein	Substrate	Sample size μl	Completion time (min)	Method of visualization	Figure in Text	Method reference
Lactic acid dehydrogenase (LDH)	Cellulose acetate	0.25—0.50	40	Formazan	1	2
Alkaline phosphatase (AP)	Cellulose acetate	0.50—0.75	60	Formazan	2	6
Creatine phosphokinase (CPK)	Cellulose acetate	0.50—0.75	45	Formazan	3	6
Phosphoglucomutase (PGM)	Cellulose acetate	0.50	80	Formazan	4	7
Erythrocyte acid phosphatase (EAP)	Cellulose acetate	0.50—1.0	40	4 MUP[b]	5	8
Esterase D (EsD)	Cellulose acetate	1.0	30	4 MUAc	6	9
Glucose-6-phosphate dehydrogenase (G-6PD)	Cellulose acetate	0.25	30	Formazan	7	10
6-Phosphogluconate dehydrogenase (6-PGD)	Cellulose acetate	0.50	30	Formazan	8	11
Adenylate kinase (AK)	Cellulose acetate	0.25	30	Formazan	8	11
Adenosine deaminase (ADA)	Cellulose acetate	0.25	30	Formazan	8	11
Glutathione reductase (GsR)	Cellulose acetate	0.5 —1.0	45	Formazan	9	11
Glutamic pyruvic transaminase (GPT) (GlPT)	Cellulose acetate	0.25—0.5	60	Formazan	10	11
Hemoglobin (Hb)	Cellulose acetate	0.25	20	O-dianisidine	11	2

Table I. (Continued)

Enzyme/Protein	Substrate	Sample size µl	Completion time (min)	Method of visualization	Figure in text	Method reference
Bisalbumin	Cellulose acetate	0.25	20	Ponceau	12	1
Group specific component (Gc)	Cellulose acetate	0.25	40	IMF[d] + Ponceau	13	12
Haptoglobin (Hp)	Acrylamide gel	1.0 —3.0	180	O-dianisidine		13
Haptoglobin (Hp)[e]	Cellulose acetate	0.5	45	O-dianisidine		14
Transferrin	Cellulose acetate	0.25	20	IMF[d] + Ponceau		12
Ceruloplasmin	Cellulose acetate	0.25	20	IMF[d] + Ponceau		12
Alpha-1 antitrypsin	Cellulose acetate	0.25	20	IMF[d] + Ponceau		12
Lipoprotein (Lp)	Agarose gel	0.5 —1.0	180	Sudan Black B		6
Lipoprotein (Lp)	Cellulose acetate	0.25—0.5	60	Ozone + PAS[f]		15

[a] All the constituents listed are determined routinely in this laboratory.

[b] 4-Methyl umbelliferyl phosphate. The zones are visualized as the fluorescence of 4-methyl umbelliferone.

[c] 4-Methyl umbelliferyl acetate. The zones are visualized as the fluorescence of 4-methyl umbelliferone.

[d] Immunofixation.

[e] This method used in the estimation of haptoglobin-hemoglobin binding capacity.

[f] Periodic-acid-Schiff.

SEROLOGY SECTION PROGRAM

SOUTHERN ASSOCIATION OF FORENSIC SCIENTISTS

AUGUST 25, 26, & 27, 1977

SHERATON TWIN TOWERS, ORLANDO, FLORIDA

PALM BEACH ROOM - CONVENTION CENTER
FLORIDA TECHNOLOGICAL UNIVERSITY

hursday, August 25, 1977

1:00 - 1:15 PM	Introductory Remarks - Palm Beach Room Steve Platt, Chairman
1:15 - 5:00 PM	Workshops A. Cellulose Acetate Electrophoresis - FTU Dr. Ben Grunbaum B. Isoelectric Focusing - Palm Beach Room LKB Instruments

riday, August 26, 1977

1:00 - 5:00 PM	Workshops A. Cellulose Acetate Electrophoresis - FTU Dr. Ben Grunbaum B. Isoelectric Focusing - Palm Beach Room LKB Instruments

aturday, August 27, 1977

9:00 - 9:45 AM	"The Concept of Assertive Testimony in Serology Cases" Richard Tanton, Palm Beach County Crime Laboratory, West Palm Beach, Florida
9:45 - 10:30 AM	"New Methods in Haptoglobin Phenotyping" Kevin Noppinger and Rodger Morrison, FTU Forensic Science Program, Orlando, Florida
10:30 - 11:15 AM	"Making Your Own Acrylamide Gradient Gels for Haptoglobin Electrophoresis" Jan Estes, Tennessee Crime Laboratory, Donelson, Tennessee
11:15 - 11:45 AM	"Group Specific Component(GC)- Application of the Grunbaum Immunofixation Procedure and Observation of a Possible Rare (GC) Variant" Jim McNamara and Rodger Morrison, FTU Forensic Science Program, Orlando, Florida
11:45 - 12:00 Noon	"Roundtable Discussion Topics" A. Origin Determinations - Anti-Sera Problems B. Electrophoresis Problems

Southern Association of Forensic Scientists

Fall Meeting - 25,26,27 August 1977

Orlando Sheraton Twins Towers, Florida

hursday, 25 August

12:00 PM - 1:00 PM	Registration in Convention Center Lobby
1:00 PM - 5:00 PM	Workshops Broward Room (1) "Micro-Crystalline Drug Analysis" Mr. Joe Koles, DEA Palm Beach Room (2) "Isoelectric Focusing" LKB Instruments (3) "Cellulose Acetate Electrophoresis" Dr. Ben Grunbaum
5:30 PM - 7:30 PM	Registration in Convention Center Lobby

Friday, 26 August

8:00 AM - 8:45 AM	Registration in Convention Center Lobby
8:45 AM - 9:00 AM	Opening of Meeting - Gold Coast Room
9:00 AM - 9:30 AM	Welcome by William A. Troelstrup, Executive Director, Florida Department of Criminal Law Enforcement
9:30 AM - 9:45 AM	Break
9:45 AM -11:45 AM	General Session - Gold Coast Room (1) "Summary of Garrison Case" Ray Herd, Arthur Hume (2) "Operating Principles of Color Xerox" Jim Kelly
11:45 AM - 1:00 PM	Lunch
1:00 PM - 3:00 PM	Section Meetings
3:00 PM - 3:15 PM	Break
3:15 PM - 5:00 PM	Business Meeting - Gold Coast Room
6:30 PM - 7:30 PM	Happy Hour - Lake Osceola Room
7:30 PM	Banquet - Seminole-Orange Room

Saturday, 27 August

8:00 AM -10:00 AM	Section Meetings
10:00 AM -10:15 AM	Break
10:15 AM - Noon	Section Meetings
12:00 Noon	Meeting Closes

TO: Advanced Technology Operation
Beckman Instruments Inc.
Anaheim, California
Attn: Mr. John Walsh

3rd INTERIM PROGRESS REPORT
1 through 30 September 1977

Subcontract: Z-847905-6
Subcontractor: University of California
Berkeley, California 94720
Submitted by: B.W. Grunbaum
Principal Investigator

30 September 1977

Introduction

On September 15, 1977, I requested certain modifications in the Technical requirements of the subcontract to permit some possibility for a usable Floodstain Analysis System. (See letter to Jack Walsh, ATO, Beckman. Sept. 15, 1977). As yet, those proposals have not been negotiated. The following report gives details of the progress of work according to the subcontract now in effect, and also explores possibilities for development of a more effective Bloodstain Analysis system. I want my recommendations to be clearly on record.

Methodology

Two major improvements in methodology for typing Hp and GLO-I have been accomplished during the month of September.

Hp. Using a Nanophor type and size gel plate, several 6% acrylamide gels (non gradient) are easily prepared for use the same day or on subsequent days. The gel thickness is 1 mm. At first blood samples were placed with a fine capillary pipet into slots that had been cut in the gel. Later, we found it possible to inject 8 samples simultaneously into gel by means of a Beckman Instruments Inc., 8 sample plastic applicator. This is not the best sample application method, it proves quite adequate. The present plate will accommodate 12 samples.

In a number of experiments, we tried a range from no cooling at all to cooling at about 5°C. The best separations are obtained by cooling with tap water while electrophoresing for 45 to 60 minutes. It was also found, as expected, that staining time is considerably shortened. This is because of the ready penetration of the o-dianisidine stain into the 1 mm gel, as compared to the rate of penetration into the 3 mm gel which is used with the Beckman Microzone system. The longer staining time is required when using the Beckman system because the specimen itself runs in the geometrical center of the gel

and is insulated with about 1 mm clear gel on each side. Also, because the o-dianisidine is not readily soluble in aqueous media, the amount of stain penetrating through the gel becomes smaller as it reaches the center of the gel. The total analysis time for Hp has now been reduced to approximately 90 to 120 minutes, as compared to the 16 hours needed for the Hp method described in my progress report to Beckman, January 10, to July 31, 1977. Immunofixation on the thin acrylamide gels for Hp was also shown to be successful.

GLO-I. A new method using 6% acrylamide gel in a nanophor type and size gel plate, proved very satisfactory for the clearcut separation and typing of the three common variants of GLO-I. Both fresh blood and bloodstains have been used. A few minor technical problems will be worked out with the availability of proper gel cutting sample application tips. As with Hp, this plate will also carry 12 samples.

NOTE: This laboratory has been and is engaged in methodology development for genetic variants apart from the Bloodstain Analysis System (BAS) subcontract. It is difficult sometimes to separate which research is done for which project. Generally, since other developmental projects are much more extensive and have been going on prior to the BAS Project, the latter is in a position to benefit from the research results. The methodology reported here on Hp and GLO-I falls into this category.

SIMULTANEOUS ANALYSES

Development of Setups

In accord with a strong recommendation from Beckman ATO, all Bloodstain Analysis Project effort in this laboratory has been concentrated upon use of starch gel-agarose gel for combined analyses. The blood constituents which have been selected for analysis have been grouped for probable compatibility in a three setup system. The first group, PGM, EsD, and GLO-I, can now be determined simultaneously on a single elecrophoretic substrate plate. With the second group, EAP, AK, and ADA, several problems remain to be resolved. The third proposed group would pair Hp and Gc. There is some doubt that it will be possible to work out a simultaneous determination of these two proteins on a single setup. An alternative would be to determine these genetic markers separately, which would, of course, require an additional setup. See attached report by Brian Wraxall.

System Development

Should the simultaneous analysis for the eight selected genetic systems in bloodstains prove feasible on three or four setups, it will be necessary to start, at once, to integrate equipment, reagents, instructions, and associated materials into a workable system for the feasibility demonstration tests. According to the contract (Task 5.2.2-System Development) it is my responsibility to recommend equipment for this stage of the Project, which will be provided as CFE. Earlier this month, I requested three Nanophors, but have had no response. I will need either these instruments or three Microzone units modified to my specifications in order to procede with System Development.

As soon as this equipment is available, this laboratory will adapt the first setup (PGM, EsD, GLO-I) for simultaneous electrophoresis on one of the units. If and when the other setups using gels are worked out, they too will be integrated into the system.

When the equipment becomes available, I will also test my hypothesis that simultaneous analyses of several polymorphic systems are better when overlays are placed parallel rather than perpendicular to the electrophoretic axis (See Beckman BAS Proposal). This innovation should avoid some of the difficulties that arise because of inconsistency in the quality of gels.

It is difficult to reproduce the exact consistency of gels from batch to batch (See Grunbaum, Ref. 1). This is particularly true with agarose gel.

It is possible that it will be recognized that the Aerospace Technical Requirement of "three electrophoretic substrate plates and no more than three cells" is not really practical, will compromise the other technical requirements, and will not serve the best interests of the forensic laboratory. In such an eventuality, it would be advisable to be able to offer a bloodstain system that is superior to the proposed starch gel-agarose gel methodology, and which holds promise of better fulfiling all Technical Requirements except the three "setups."

There are several arguments in favor of the new methods. First, the confirmation tests (Monthly Progress Report BAS, September 8, 1977) clearly demonstrate that the newly developed methods for bloodstain typing using cellulose acetate yield as good results as the old-fashioned methods using gels. Secondly, it is now possible to perform all these tests using only two kinds of supporting media. Acrylamide gel will be used for GLO-I and Hp. CAM will be used for the six others. Fianlly, the new methods will better meet the over-all contract requirements.

Two approaches suggest themselves: (1) A single Microzone cell (modified) may be used as shown below:

Order of Analysis	Substrate	Hands On	Elapsed Time
Sample Preparation	CAM	15 min.	15 min.
PGM	"	15 "	75 "
EsD	"	15 "	60 "
Ak-ADA-EAP (simultaneous)	"	25 "	60 "
GLO-I	Acrylamide gel	30 "	150 "
Hp-Gc (simultaneous)	"	35 "	120 "
	Total time	135 min. (2 h. 15 min.)	480 min. (6 h.)

Imperceptible varying amounts of trace contaminants may radically alter electrophoretic patterns as a result of electroendosmosis, which is difficult to control. There is always the possibility that overlap and too-close margins will result in loss of bands when phenotype patterns are determined in the perpendicular fashion (See Beckman's Monthly Progress Report, September 8, 1977). Thus it is likely for laboratories using agarose gel from different sources to obtain conflicting results on the analysis of the same bloodstain specimen.

The bloodstain will be placed in the gel with appropriate standards on either side. Since the proposed setup can accommodate 12 samples, it is thus possible to arrange for the simultaneous analysis of PGM, EsD, and GLO-I (or any other group of three determiners that can use a common pH and buffer). At the end of electrophoresis, the overlays with appropriate substrates will be placed parallel to the electrophoretic axis of each variant and there is no possibility of loss of information due to overlapping orfailing to cover any of the bands, regardless of the quality and makeup of the gel.

Very little has been reported in forensic literature of any research attempts at simultaneous electrophoresis using single "setups." However, a recent paper (See ref. 2) describing such research concludes with the following remarks:

"There are several precautions which should be stated when doing multiple enzyme systems. First, there are the considerations of pH and ionic strength which are not necessarily optimal for each enzyme studied. Second, in any multiple enzyme system, the rarer variant banding pattern of any one of the enzymes that overlaps into the development region of a second enzyme will not be visualized. Thus, multiple enzyme systems might best be used as a rapid screening test to observe basic enzyme variants." Would it not be better to analyze for single constituents in the first place and do it right under optimal conditions?

It will be very easy to repeat, if necessary, any one or all of the analyses within the time limits allotted by Aerospace requirements. (2) Using two or three cells, it is obvious that speed of analysis will be increased, and more flexibility will be afforded.

This approach has recently been tested as "Technology Transfer" during a workshop sponsored by the Southern Association of Forensic Scientists. See attached letter from the US Army Criminal Investigation Laboratory and my reply.

Comment

By the time the final report of the BAS is completed, the arguments concerning the use of starch gel vs. cellulose acetate may be irrelevant. It is now known that separation of proteins in a linear pH gradient produces an unusually high degree of resolution by separating proteins according to their isoelectric point. Electrofocusing on polyacrylamide gel, not only results in many more electrophoretic zones, but each zone is highly concentrated as an inherent feature of the technique. Thus, for instance, in the EAP system, Kaczmarek, (see Ref. 3) using a highly purified enzyme, showed 21 isoenzymes for a given phenotype, while conventional electrophoresis shows only about two or three (so called primary, secondary, tertiary bands).

Using isoelectrofocusing for typing the common PGM variants, Bark et all (see Ref. 4) demonstrated 10 new variants. They thus increased the discrimination probability of PGM from about 1 in 2 to 1 in 4 individuals. This is of tremendous significance when examining bloodstain evidence. Grunbaum in 1975, (see Ref. 5) working with dried bloodstains found about 60 bands (zones) after isoelectric focusing. On the basis of this finding, a system was proposed which would make it possible to determine 5 to 10 genetic systems simultaneously. Subsequently Burdett and Whitehead (see Ref. 4) reported simultaneous separations of several polymorphic systems.

It should be noted that in the Beckman Technical Proposal, Part II, Electrophoretic Bloodstain Analysis System, pp. 11-14, the proposed general approach recommended the evaluation of the technique of isoelectric focusing to determine its potential for methodologic simplification. It is unfortunate that Aerospace chose to delete all studies related to the use of isoelectric focusing for electrophoresis.

The Beckman proposal also included an approach for the definition of "markers" which would indicate whether components had seriously degraded (p. 17). Aerospace deleted all enzyme marker studies related to determination of age of stain for ascertaining whether the stain can be easily analyzed by electrophoresis. Independent of the BAS, this laboratory has carried out the marker system studies, with useful results. Using the highly sensitive and specific electro-immuno-precipitation method, it is possible to assess the relative amounts of Hp and Gc remaining in a bloodstain. This test is carried out on cellulose acetate using the unmodified Beckman Microzone cell. If no Hp or Gc shows up in-these tests, phenotyping by electrophoresis is probably of no use. If, on the other hand, Hp and Gc are present to any extent the chances are that the stain is relatively fresh or well preserved and warrants the complete analysis for all variants.

References

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5. B.W. Grunbaum, Some new approaches to the individualization of fresh and dried bloodstains
   J. Forens. Sci. 21, 488 (1976)

DEPARTMENT OF THE ARMY
UNITED STATES ARMY CRIMINAL INVESTIGATION LABORATORY–CONUS
FORT GORDON, GEORGIA 30905

CIRCL-CT

20 September 1977

Dr. W. Grunbaum
Building T-2251
University of California Berkeley
Berkeley, CA 94720

Dear Dr. Grunbaum,

The workshop you held in Orlando at the SAFS meeting was most informative. The other serologists in this laboratory and I are anxious to try as many enzyme systems as possible on cellulose acetate membranes, and our Beckman Microzone system is now operational. We have reagents for EAP, EsD, PGM, G-6-PD, Pep A, AK, and human semen, and would greatly appreciate copies of your procedures for these and any other systems you have developed.

Sincerely,

Vivienne S. Stopper
VIVIENNE S. STOPPER
Forensic Chemist
Serology/Trace Evidence Section
Chemistry Division

UNIVERSITY OF CALIFORNIA, BERKELEY

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WHITE MOUNTAIN RESEARCH STATION

BUILDING T-2251
BERKELEY, CALIFORNIA 94720
23 September, 1977

Ms. Vivienne S. Stopper
Forensic Chemist
Serology/Trace Evidence Section
Chemistry Division

Dear Ms. Stopper,

Thank you for your letter of September 20, 1977. Under separate cover I am sending you copies of my procedures for EAP, EsD, PGM, Gc, and G-6-PD, for use with both fresh blood and dried bloodstains. The other procedures you have mentioned are still under development.

I am most interested to learn that you and the other serologists in your laboratory are planning to try the determination of enzyme systems on cellulose acetate. The methodology under development in my laboratory is of value only if it can be learned easily and put to good use in crime laboratories. I would very much appreciate receiving from you an account of your successes and difficulties in use of these procedures. I would also welcome any suggestions you may have regarding procedures and, especially, how to make workshops more effective.

It is my feeling that the workshop in Orlando was hardly more than an introduction to the CAM procedures, and that those in attendance can not be expected to use this methodology without further instruction. I would be interested to know if you run into any difficulties, and would be most willing to help you work out any problems.

If you, or anyone from your laboratory, could arrange to visit us here in Berkeley for a few days, you would be most welcome to become acquainted with the work we are doing here and train in the methodology under our instruction.

Please let me know if I can be of any further help.

Sincerely Yours,
Benjamin W. Grunbaum
Benjamin W. Grunbaum, Ph.D.
Research Biochemist

BG:sw

Bloodstain Analysis System Project
Multisystem Status

23 September, 1977

As stated at the last program review the work on Multisystem analysis falls into three groups.

I.	At pH 7.4	PGM, ESD, GLO I
II.	At pH 5.9	EAP, AK, ADA
III.	At pH 8.3	Hp, Gc

GROUP I

This system has been conducted mainly on starch. The electrophoresis is carried out in approximately four hours after which ESD is the first to be developed. The results are read and photographed followed by the development of the PGM and GLO Systems. There is no overlap with these two systems and all rare variants can be seen. Subsequent work with agarose showed considerable promise. The addition of starch was necessary for the development of GLO so this was incorporated into the gel at 2%. Working with this system, electrophoresis time is reduced and there is considerable improvement in the separation of the GLO and ESD isozymes. There is no reduction in the separation of PGM. Approximately 65 electrophoretic analyses have been conducted to detect this combination of systems. 1 to 4 week old bloodstains have been analysed in this group, the majority of which have given readable correct results.

Problem Areas

There are very few here. The limits of use need to be determined on the following:
1. The amount of starch
2. The GLO reaction mixture
3. The ESD reaction buffer

Conclusion

This group will constitute the first set-up.

GROUP II

This system has also been conducted mainly on starch although it is hoped that it will eventually be used on agarose or agarose/starch. Citrate-phosphate buffers have been investigated in the pH range of 5-6.5. There has been a significant improvement in the separation of EAP isozymes and a small improvement in the resolution of AK isozymes. In the original system ADA was unsatisfactory in its separation. An improvement has been noted in other buffers (eg: pH 5.5) and work is continuing in this direction. Approximately 20 analyses have been conducted using this combination with many more being conducted on an individual basis using different buffers.

Problem Areas

1. The ADA reaction mixture is giving continual problems which is either due to impure chemicals or to the reaction being conducted outside its pH/molar conc. range.
2. There is a slight overlap between AK and EAP where the rare variant AK 2-1 may be missed. This may be overcome by a change in pH or by developing the isozymes separately.
3. The individual enzymes have different sensitivities to reducing agents which may cause problems.

Conclusion

This group has great promise and will form the basis of the second set-up.

GROUP III

This group is the most difficult and requires the most work. The first approach was to detect both proteins by antigen-antibody crossed electrophoresis (AACE). The results are very encouraging with all types of Gc and Hp being separated. However, while the separation of Gc is excellent, the Hp phenotypes (due to very little charge differences between them) do not separate very well. There is also a certain amount of manual dexterity required when using this system.

The second approach now being conducted is to try to separate the proteins on acrylamide, detecting the Hp with o-tolidine and the Gc by immuno-fixation. There are many combinations of buffers and acrylamide concentration and so it may be some time before the right combination is found.

It is possible to phenotype both Gc and Hp on acrylamide using serum samples but on stains there is an excess of Hb which tends to mask the banding. Approximately 20 analyses have been conducted on acrylamide and about 12 by AACE.

Problem Areas

1. Using AACE there is an interaction between the two antisera. Antisera from different manufacturers can be tried.
2. Finding the right combination of buffer and acrylamide concentration which will enable both Hp and Gc to be separated.
3. The excess HB which occurs in bloodstains causes serious problems in Hp typing.
4. As mentioned earlier, Gc protein is unstable and therefore it will probably only be detected in 50 to 60% of all bloodstains.

Conclusion

The group has promise but it requires a great deal of work before a decision can be made. However, if this combination does not work then individual systems can be used, but it would require an extra set-up.

OVERALL CONCLUSIONS

Seven stains submitted to the laboratory from the Forensic Biology Study Group were analysed by Multisystem analysis. One application of stain gave correct results for all stains in PGM, GLO I, and ESD. A second application gave correct results in EAP and AK. Analyses for ADA, Gc, and Hp were not carried out.

Multisystem analysis has a very high potential in this program with a very good possibility of being able to analyse four week old bloodstains with a discrimination probability of 1 in 200 using only three set-ups.

Brian Wraxall
Beckman Consultant
Bloodstain Analysis System Project

cc: E.W. Grunbaum ✓
J. Bordeaux
J. Walsh

APPENDIX

SECTION B

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WHITE MOUNTAIN RESEARCH STATION

BUILDING T-2251
BERKELEY, CALIFORNIA 94720

20 July 1977

Mr. John Walsh
Director, Life Sciences
Advanced Technology Operation
Beckman Instruments, Inc.
1630 South State College Blvd.
Anaheim, California 92806

Dear Jack:

I am writing in reference to the meeting on the Bloodstain Analysis System which took place at the White Mountain Research Station in Berkeley on June 20, 1977. As principal investigator, I expected a copy of the minutes of this meeting to be sent to me by the Aerospace Corporation. Although this has not been done, I have seen the copy sent to Del Re and Harmor.

The minutes indicate that five representatives from the Aerospace Corporation were in attendance. Five Beckman people were listed as present, including the two consultants, Brian Wraxall and Mark Stolorow, who are working as guests in my laboratory. The University of California was represented by two technicians who work part-time on the Bloodstain Analysis System project at my discretion.

I wish to make several comments regarding this meeting and conclude with a request.

1. This laboratory was not represented at the June 20 meeting. In retrospect, I feel that any Aerospace-Beckman-U.C. meeting should have been postponed until my return from Europe. Benny Del Re and Gary Harmor, who attended the meeting, do not have the authority to represent either this laboratory or the University of California in a meeting of this kind. They are technicians employed by the University of California who work under my direct supervision and spend only part of their time on the Bloodstain Analysis System program, at my discretion.

2. According to the terms of the subcontract between the University of California and the Beckman Corporation, it is the responsibility of the University laboratory to select the methods to be included in the Bloodstain Analysis System. It is Beckman's responsibility to test this system after it is presented by the University laboratory. I was astounded to read in the Minutes that the two Beckman consultants had announced decisions concerning methodology. As one example, Brian Wraxall discusses conclusions regarding the various substrates. They have not produced sufficient experimental evidence to support such decisions. I must go on record as saying that I do not agree with the statements that these Beckman consultants have made. I must challenge their right to make any decisions in regard to methodology. Their opinions are welcome, but the final responsibility rests upon me, as principal investigator and representative of the University of California.

It is my opinion that any comments and evaluations made by Beckman consultants should be made freely to the proper Beckman representatives. However, reports to Aerospace should first be cleared with both the University of California and Beckman.

3. Finally, I must protest the "flap" regarding the Nanophore. According to the agreement between NASA and LEAA, two Nanophores were to be left in this laboratory for four months, and one of these was to be left here for an additional seven months. Before I left for Europe the methodology using cellulose acetate membranes was thoroughly tested on the Nanophore. As you will remember, I presented some of the excellent results at the Aerospace-Beckman-U.C. meeting here in May. The first unit was returned to NASA at their written request for additional developmental work just before my departure. It was not needed in the laboratory. While the unit remaining in the laboratory was not equipped for use with cellulose acetate, it was ideal for use with starch gel and other gel media. I had requested, and the two Beckman consultants had agreed to test the starch gel methods with the Nanophore during my absence. They did not do so, but the reason was not that the Nanophore was not available.

I am requesting that you set the record straight with the Aerospace Corporation. First, nothing reported at the June 20 meeting by the Beckman consultants is to be interpreted as authorized by me, as director of this research, or the University of California as subcontractor. Secondly, I absolutely disagree with most of the findings of the Beckman consultants and I challenge their right to make any decisions regarding methodology.

At this time, the work in this laboratory is on schedule and is progressing in a highly satisfactory manner. I feel that it will be in the best interests of the University of California and of Beckman that, in the future, we resolve differences of opinion prior to any meetings with Aerospace. It is understood that I take full responsibility for decisions concerning methodology. It is in Beckman's interest to make it clear that their consultants are serving as advisors, not decision-makers.

I will be interested to receive your response to the thoughts I have expressed in this letter. Especially, I hope that we will be able to present to the Aerospace Corporation a clear statement regarding obligation and responsibility, and that it be clearly understood that I do not in any way endorse the findings and conclusions as recorded at the June 20th meeting.

Sincerely yours,

B. W. Grunbaum

EWG:emn

Berkeley on 10 Aug 1977

In reference to the official minutes of the Bloodstain Electrophoresis Program Review that was held at the White Mountain Research Center, University of California, Berkeley, 20 June, 1977.

First, I request that it be put in the record that since I was not present and had delegated no one to take my place, the University, as subcontractor, was not represented.

Secondly, I feel it appropriate to remove from the minutes certain remarks by Brian Wraxall concerning a sample analyzed as part of an OCJP project. As Mr. Wraxall is not involved in any way in the OCJP project and does not have my authoration to analyze OCJP samples, his remarks to a joint meeting of Aerospace and Beckman represent a violation of the hospitality of this laboratory. Further, his comments and conclusions are incorrect, misleading, and show a lack of understanding of the way in which OCJP data are handled.

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August 18, 1977

University of California
White Mountain Research Station
Berkeley Campus, Bldg. T-2251
Berkeley, CA 94720

Attention: Dr. B. W. Grunbaum

Dear Ben:

I have enclosed copies of the work plan we agreed on at our meeting of August 16. As we have all recognized it will be necessary to use the limited time and manpower most effectively if we are to solve the formidable problems remaining on this program.

Identifying and assigning specific tasks to members of your team will assure that proper priorities are established and progress easily monitored. In any development project, the unexpected must be accommodated. I suggest we do this by reviewing and updating the task assignments on a weekly basis.

I am pleased with the spirit of cooperation and dedication which is being expressed by you and your University employees and our consultant, Mr. Wraxall. I am confident that your combined efforts will be successful in meeting our program objectives.

Sincerely,

Jean Bordeaux
Program Manager.

JB:gw

cc: R. Shaler
The Aerospace Corporation

B. Wraxall
BII Consultant at UCB

Enclosure

MAJOR TASKS - SYSTEM DEVELOPMENT

1. Run confirmation tests.
2. Review substrate selection.
3. Review system variables.

   Establish groups of constituents on:

   - Starch
   - CAM
   - Acrylamide
   - Agarose

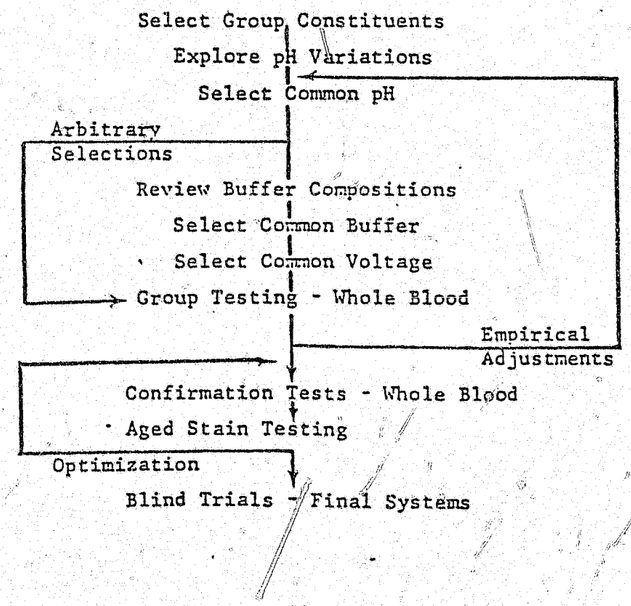
4. Establish limits for process variables.

   - pH
   - Buffer Composition
   - Separation Time
   - Separation Distance
   - Voltage/Amps

5. Review data and select compromise values for the process variables for each group of constituents.
6. Run a series of tests with each group to determine ability to achieve accurate phenotyping.

   - Whole Blood
   - Stains

TYPICAL SYSTEM DEVELOPMENT LOGIC NETWORK



Tasks	People	Time Start	Time Complete
Confirmation Tests	Brain, Gary	8/15	8/19
Review Substrate Selection	Ben		
Establish Constituent Groups	Ben		8/19
Trip	Ben	8/22	8/29
Group Analyses			
1. PGM, AK, EsD - CAM	Gary	8/17	8/29
2. EAP, ADA, GLO I - Starch	Brian	8/17	8/29
3. H_p, G_c - CAM	Ben	8/22	10/15
- Agarose	Brian	1.P.	10/15
4. Other Groups TBD	Ben	9/1	
(PGM, EsD, GLO I)			
(EAP, AK, ADA)			
(PGM, EsD, AK, ADA, EAP)			

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September 6, 1977

B. W. Grunbaum, Ph.D.
University of California
Building T-2251
Berkeley, California 94720

Dear Dr. Grunbaum:

A great deal of effort has been expended during the System Definition Phase of the Bloodstain Analysis Program to study and select optimum media for the analysis of aged, dry bloodstains. For most of the analyses, two approaches have been considered in parallel -- the use of starch gel and cellulose acetate.

Our program schedule dictates that the System Definition Phase be concluded to permit focus of our attention on the System Development Phase. It is therefore necessary at this time to select the approach offering the greatest promise of meeting contract objectives.

Your past and current activities demonstrated the adequacy of cellulose acetate for the analysis of fresh blood. We have great doubt, however, that all contract objectives can be satisfied using cellulose acetate without significant additional effort.

On the other hand, we feel that adequate evidence has been presented to show that the starch gel-agarose gel method for combined analyses offers great promise for the isoenzymes under consideration -- GLO-I, PGM, EsD, EAP, AK, and ADA. Blind trials demonstrated that starch gel was superior to cellulose acetate for some of these and at least equivalent for the others.

It may be that continuation of the System Definition Phase would result in a cellulose acetate based system that would be superior to one based on starch gel. However, the severe funding limitations preclude this course of action. Continuing investigations into the use of cellulose acetate for the analysis of the above isoenzymes constitutes a diversion of needed resources from the more promising approach, and we strongly recommend discontinuation of such studies now.

ellulose acetate is still a candidate for the combined analysis of
c and Haptoglobin, using immunofixation techniques for localization
and identification. Optimization effort should continue for the
analysis of these two proteins.

We feel that this approach should be implemented as soon as possible.
If you have any questions, please do not hesitate to contact me.

Sincerely,

John M. Walsh

J. M. Walsh
Manager, Life Sciences

dm

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BERKELEY, CALIFORNIA 94720

24 October 1977

Mr. Jean Bordeaux
Program Manager
Bloodstain Analysis System Project
Advanced Technology Operations
Beckman Instruments, Inc.
1630 South State College Blvd.
Anaheim, California 92806

Re: Official Minutes, Program Review Meeting,
Electrophoretic Bloodstain Analysis
Beckman/University of California
3 October 1977

Dear Jean:

Will you kindly direct Dr. Shaler, Director, Forensic Sciences, to make the following corrections in the Official Minutes?

1. The heading "Sequential Analysis (Parallel Separation)" is misleading in that it suggests that somehow these terms are synonymous. The paragraph which follows does nothing to relieve this basic misapprehension.

In the handout you prepared for this meeting (which is attached to the Minutes) and in my oral presentation at the meeting, two distinct and separate approaches were described as possible alternatives to the system development which has been selected by Beckman. Sequential analysis is a system for making all analyses on a single cell. Parallel Separation is a method for simultaneous analysis on three "setups" using either agarose/starch gel or cellulose acetate membranes.

2. This section entitled "Sequential Analysis (Parallel Separation)" also states that "this effort will be continued until such time as the simultaneous analysis system has been fully developed and the potential for system compromises has been eliminated".

As you well know, all effort in this laboratory is now directed toward the agarose/starch gel system of simultaneous determination. As you mentioned in your Monthly Progress Report, dated October 7, 1977, a letter recommending this direction was sent to me from Beckman on September 6th. This letter made it quite clear that budgetary limitations did not permit the pursuit of alternate approaches. Since receipt of that letter, I have assigned a full-time technician to work with Beckman's Consultant in support of this recommendation. As you know from your frequent visits to this laboratory and from my written reports, no effort is being made in this laboratory to develop alternate approaches as part of the BAS Project. You yourself clearly indicated in your monthly report that the alternate work which was described is the product of other programs under way at the White Mountain Research Station.

3. The section entitled "Equipment Selection" contains the statement that "pricing data on the Potential Equipment has been collected". I do not recollect that such data was presented at the meeting. If I am in error, will you kindly refresh my memory by supplying me with the data. If I am not in error, the Minutes should be corrected accordingly.

4. The Minutes conclude with the statement that "Draft copies of both the Feasibility Demonstration Test Plan and the Crime Laboratory Demonstration Test Plan will be submitted to Aerospace by 24 October 1977." I do not remember discussion and agreement concerning these changes in schedule at the Program Review Meeting. If such changes had been discussed, I would have raised immediate objection, since this laboratory is under contract to support preparation of both these plans. I suggest that the remark I have quoted be deleted so that there will be no suggestion that either this laboratory or Beckman Instruments failed to comply with an agreement.

5. Finally, I would like an acknowledgement in the Minutes, that I, as sole representative of the University of California, presented research results and other approaches, for the purpose of expressing a minority dissenting opinion. The Parallel method for simultaneous analyses, using three setups, was offered as a system now available and superior to the approach chosen by Beckman for development for simultaneous analysis. The sequential method was presented as a system which is more economical, more practical, and more in keeping with the needs of the U.S. forensic laboratory than the system which Beckman has chosen to develop.

6. I am chagrined that the substance of my report at the Program Review Meeting was either ignored or grossly misunderstood in the official Minutes. I would like acknowledgement in the Revised Minutes that I, as sole representative of the University of California, described in detail methodology for dried bloodstain analysis which has been developed in this laboratory and which were not accepted for System Development. It is only because I am firmly convinced that Beckman Instruments did not choose the best available methodology for Systems Development that I made the effort to register a minority opinion, and I believe the official record should reflect this dissent.

I did not offer the parallel method for simultaneous analyses and the sequential system of analyses as <u>possible</u> alternatives if the system of first choice was unsuccessful. On the contrary, I offered them for <u>immediate</u> adoption. The parallel approach would immediately solve the three "set-up" problem. On the other hand, the sequential approach would result in a system that is more economical, more practical, and more in keeping with the needs of the U.S. forensic laboratory than any three "set-up" system.

Sincerely yours,

B. W. Grunbaum

BWG:emn

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1630 SO. STATE COLLEGE BLVD., ANAHEIM, CALIFORNIA 92806 • TELEPHONE: (714) 997-0730 • TWX 910-592-1260 • TELEX: 06-78413

October 27, 1977

Dr. B. W. Grunbaum
White Mountain Research Station
University of California
Building T-2251
Berkeley, California 94720

Re: Official Minutes, Program Review Meeting, Electrophoretic Bloodstain Analysis, Beckman/University of California, 3 October 1977

Dear Ben:

In response to your letter of October 24th, I am forwarding a copy of your letter to Dr. Shaler with my comments as indicated in the following paragraphs.

As you have pointed out, there are points in the minutes which are not correct. Taking these points one by one as enumerated in your letter, your item one is correct and the minutes should be clarified accordingly.

Item two is also correct except that 25% of your time is available for work on some specific items such as the serum proteins.

You are correct in item three in that no pricing data was presented. We have not completed a compilation of prices as yet.

In item four, no commitment was made at the program review to supply either plan to Aerospace by October 24th.

As to item five, we can make it clear that you presented the alternative approaches of both sequential and parallel analyses. However, your comments of the last two sentences of item five are matters of opinion which have not been substantiated by test results and, as you are well aware, are not in accordance with the statement of work.

Again, in regards to item six, there is no reason for not clarifying the minutes to show your dissenting opinion. But, as noted above, your comment that the parallel approach (on CAM) would immediately solve the three set-up problems is not substantiated by test data.

There is no question that it would be desireable to continue development of the CAM methodology. However, under this program it would require a change in the statement of work and additional funds.

Sincerely yours,

BECKMAN INSTRUMENTS, INC.

Jean Bordeaux
Program Manager

jw

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2 November 1977

Mr. Jean Bordeaux
Program Manager
Bloodstain Analysis System Project
Advanced Technology Operations
Beckman Instruments, Inc.
1630 South State College Blvd.
Anaheim, California 92806

Dear Jean:

I have received your letter of October 27, 1977, in reference to the Official Minutes of the Program Review Meeting, October 3, 1977. Thank you for sending a copy of my letter to Dr. Shaler as I requested.

I have taken note of your comment to Dr. Shaler that 25 percent of my time is available for work on this project. This is undoubtedly a useful clarification to Item 2 of my letter.

Your comments in reference to Items 5 and 6 reflect a certain misunderstanding, which I feel obliged to remark upon in order to set the record straight.

First, there seems to be a suggestion that I am insisting on continuing the development of CAM methodology and must be overruled in consideration of budgetary limitations. In fact I have made no proposal to continue development of CAM methodology as part of the BAS project beyond the conclusion of the System Definition Phase. I have suggested the combination of certain already-developed CAM methodology and certain gel methodology into a bloodstain analysis system.

Secondly, I note that there is still some confusion regarding the Parallel Approach. You state that my "comment that the parallel approach (on CAM) would immediately solve the three set-up problems is not substantiated by test data". I must point out to you that the words in parenthesis "on CAM" are yours, not mine. As you may remember, we demonstrated the parallel system to you in this laboratory, on gel, prior to the last Program Review Meeting. I described this approach at that Program Review Meeting, to illustrate how it would work to facilitate a three set-up system using the agarose/starch gel methods that Beckman has chosen. I described this parallel approach for use with gel in my Interim Report, dated September 30, 1977. Of course, this parallel approach can also be used with cellulose acetate, but this was not my proposal. Your remark that my claim is "not substantiated by test data", suggests that some tests have been made, with negative results. This, of course, is not true. This approach would indeed meet the requirements of the Statement of Work, and I am prepared to support my claim with a laboratory demonstration, utilizing the starch gel/agarose gel methods favored by Beckman.

National Institute of Justice
United States Department of Justice
Washington, D.C. 20531

I am indeed well aware that my suggestion for sequential analysis on CAM and agarose/starch gel is not in accordance with the statement of work. However, I feel it my responsibility to present alternate proposals to Beckman and Aerospace.

Finally, my proposals are something more than unsubstantiated opinions. I must assume that my participation in this project came about as a result of my many years of work in this area and, consequently, my remarks may be regarded, at the very least, as "expert". In fact, any statements or claims that I have made have been checked out thoroughly in the laboratory, and I am prepared to substantiate them.

I hope I have cleared up any misunderstandings concerning the items in my letter to you of October 24, 1977. Please send a copy of this letter to Aerospace Corporation.

Sincerely yours,

B. W. Grunbaum

BWG:emm

APPENDIX

SECTION C

MONTHLY PROGRESS REPORT

BLOODSTAIN ANALYSIS SYSTEM

Subcontract 67854

March 8, 1977

Prepared for:

The Aerospace Corporation
Suite 4040, 955 L'Enfant Plaza, S.W.
Washington, D.C. 20024

Beckman INSTRUMENTS, INC.
ADVANCED TECHNOLOGY OPERATIONS
ANAHEIM, CALIFORNIA 92806

This is the second monthly progress report of the Electrophoretic Bloodstain Analysis Program.

This report reviews the work performed during the month of February, 1977.

The major tasks for this period were to establish a source of blood samples and the procedure for the 4-week aging test; finalize the arrangements for Mr. Wraxall's visa; and continue optimizing analytical procedures for enzymes being investigated. Final arrangements were made to procure all of the necessary hardware for the definition phase of the program. The highlights of these tasks and details of other activities supporting the program are presented in the following paragraphs.

Candidate Analytes

Major emphasis of the program at this time is the optimization of the analytical procedures for selected constituents. Last month a group of 10 constituents were reported. These ten, repeated here for convenience - PGM, AK, ADA, EAP, EsD, Hb, GC, G-6-PD, 6-PGD, and Hp form the nucleus of the candidates thus far. They have demonstrated good resolution with fresh blood and, if stable for 4 weeks, would provide discrimination better than 1 in 200. The 4-week aging tests have now been started on these constituents and preliminary data will be available by the end of the coming month. In addition, we are continuing investigation of GSR and GPT. Results of testing of these two constituents are promising but the methods have not yet been developed to the point where the procedures for them are completely satisfactory. GPT is one of the more desirable phenotypes but it has been reported previously that it is not stable. However, we are optimistic that the technique we are developing for its analysis will overcome some of the problems that have been noted in earlier efforts to analyze for this constituent after it has been aged. Work is also continuing with carbonic anhydrase II and peptidase A. Work has not

yet progressed to the point where either of these can be judged satisfactory or unsatisfactory.

The most exciting one of the new constituents is of course Glyoxylase. With a discrimination factor of about 35, it would enhance the overall discrimination capability greatly if it can be successfully developed. Work has commenced with this constituent. A procedure has been tried on cellulose acetate which appears to work but resolution is not yet satisfactory. It is possible that the reagents used contain impurities that are causing problems. The work to determine if this is true is laborious and time consuming.

Mr. Wraxall has reported success with this constituent in fresh blood. After he has joined the team and has contributed his thoughts, the approach can perhaps be refined so that it will be satisfactory with aged blood.

The search for additional constituents will continue for at least another two months. The probability of success in establishing an entirely new constituent is quite low. The field has been surveyed now for several years. Some groups have dedicated their efforts to this particular area and in the past year very little new data has been forthcoming. We will continue to monitor possible sources for the recognition of new constituents and will immediately investigate any coming to our attention.

Blood Samples

One of the major areas of concern was a source of blood samples to support the program. Originally we had thought to obtain pint samples from a local blood bank. At first it appeared that this was desirable and could be accomplished. However, the blood banks have been reluctant to provide samples in these volumes. Therefore, we have reviewed our requirements and have determined that the present sample supply for the OCJP program will in fact be adequate for our needs. These samples come in daily. During the course of the day the samples can be analyzed and screened for significant variants. Samples which appear to be of importance to our program can be immediately identified

and stains made on glass slides for 4-week aging. Using the OCJP samples will permit us to have aging tests going on in a continuous manner as opposed to batch sampling every 2 to 3 months as had been planned earlier. These samples come in small plastic tubes and contain ACD as an additive.

Storage

The 4-week aging tests will be controlled by placing the glass slides in a cabinet which has trays that permit the slides to lie flat. The slides can be coded for positive retrieval and easily controlled for the 4-week aging period. The cabinet containing the slides will be simply placed in an environmentally-controlled room where the ambient temperature will vary only ± 2 to $3^{o}C$.

Supporting Media

We have delayed ordering equipment for starch and agarose gel separation until concurrence by Mr. Stolorow and Mr. Wraxall could be obtained. To date the work has been directed towards using cellulose acetate and acrylamide gel. Shandon and Gradipore equipment for agarose and starch gel is now on order. This equipment will be operated by both Mr. Stolorow and Mr. Wraxall in comparing the performance of their standard techniques with that equipment against results obtained from the Microzone units using cellulose acetate or as modified to use acrylamide, agarose or starch gels.

Personnel

Mr. Stolorow joined our team the first of February. During this past month, Mr. Stolorow was subpoenaed for three separate court cases, taking him away from our program for about 8 days. In addition to this absence, he also spent a day at the San Diego meeting of the American Academy of Forensic Sciences. Therefore, during the month of February a substantial amount of his time was lost, and there was a rather limited contribution to the work in developing our analytical techniques. Mr. Stolorow has become acquainted with the use of the Microzone hardware. He ran about two hundred samples with it.

The way has been cleared for Mr. Wraxall to join us at the end of February. He is expected to be at Berkeley by the first of March and, after allowing a couple of days to locate, will be effective in supporting our program.

Program Support Meetings

During the month of February, three direct meetings with Dr. Ben Grunbaum and his team were held. Close liaison has been established and will be maintained so that any problems that might impede our progress will be rapidly identified and allow prompt corrective action to be undertaken.

Work Planned for the Coming Month

At this time, the program is on schedule. Work will continue on all of the tasks as planned during this coming month. We will be planning for the second program review which is now scheduled to take place on March 15th. At this time we do not have any significant problems to resolve.

Cost Status

Technical accomplishment on the program is consistent with the expenditure of funds. The requested change in meeting location and replacement of Dr. Yoong with the two consultants have increased the projected total cost. It is anticipated that the increase can be offset by other economies during the life of the program. Please see the Monthly Cost Report for additional detail.

MONTHLY PROGRESS REPORT

BLOODSTAIN ANALYSIS SYSTEM

Subcontract 67854

April 7, 1977

Prepared for:

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Third Monthly Progress Report – Electrophoretic Bloodstain Analysis Program

This report reviews the work performed during the month of March 1977.

The major tasks for this period were to conduct a Program Review Meeting and begin testing of aged samples. The highlights of these tasks and details of other activities supporting the program are presented in the following paragraphs.

Program Review Meeting

On March 15, a program review meeting was held at the White Mountain Research Station, University of California, Berkeley. This was the first meeting at which all of the technical participants on the program were present. The responsibilities of the members of the technical team were discussed and the coordination of their activities explained. Details of the agenda are covered in the minutes prepared by The Aerospace Corporation. A copy of the minutes was received from The Aerospace Corporation and reviewed. Beckman does not have any corrections or additions to make to the minutes except to clarify that the testing of the new multizone cell is not a firm commitment.

Four-Week Aging Test

During this month, testing was initiated on samples of blood aged for four weeks. The samples as received contain up to 15% ACD. Drops of blood of approximately 25 microliters in volume were deposited on glass microscope slides and stored in a horizontal position in an enclosed box.

The samples in this series were received from eight individuals selected at random without any genetic or other restrictions. The dried blood was removed from the slide with a razor blade and reconstituted with water. Only cellulose acetate and acrylamide gel membranes on the microzone cell were used for these beginning tests. Of the 10 systems reported last month which have been successfully analyzed in fresh blood with the microzone cell, four yielded unambiguous results after four weeks aging. One other was borderline and

optimization will be required. Of the remainder, evidence of enzyme activity was apparent but improved methodology is needed for unambiguous interpretation. The four successful systems are: Eap, Hp, PGM, and AK. A fifth, ADA, could be read but needs work as noted. These five yield a discrimination probability of 1 in 23. However, the addition of only three others, Glyoxylase, Gc, and EsD, would increase the probability to 1 in 200.

Work will proceed to develop these systems and ultimately to combine them to run on three substrate plates.

System Priorities

At the program review, three groups of systems were preliminarily classified into a relative priorities based on a combination of the discrimination capability and the expected persistence of the particular system. According to the references appended, all six systems of group 1 should be sufficiently stable after four weeks aging to yield satisfactory analyses.

The classification was meant to focus attention on the systems which have the highest potential for satisfaction of program requirements. It should be emphasized that all the systems listed are under consideration and nothing is being rejected out of hand. As many systems as necessary will be included to reach the discrimination goal of 1 in 200.

Isoelectric Focusing

During the Program Review, it was noted that PGM would have a discrimination potential of 0.25 if its variants are separated using isoelectric focusing vs. 0.47 on cellulose acetate or starch electrophoretograms. Other systems may also have improved discrimination with this technique. Our present program does not include isoelectric focusing as an area for investigation. However, if it should appear that significant benefits to our program might be derived from this technique, perhaps some consideration should be given to how they might be obtained. This may be considered in a future trade-off analysis.

Comparative Testing

During the past month, a major portion of time of the Beckman consultants was devoted to the organization and setup of their trailer laboratory facilities. As explained at the Program Review, it is intended that the consultants have equipment comparable to that use routinely in their own laboratories so that direct comparisons can be employed in the selection of analytical methodologies.

Equipment from Shandon & Gradipore was obtained for this purpose. The Gradipore unit was defective in several areas, and after a considerable struggle to debug it, it has finally been returned to the manufacturer. A Pharmacia unit, which is similar in acrylamide gel capability, has been borrowed and is in use.

Many minor but time-consuming details of getting the overall operation into smooth working order have now been accomplished. Runs have been made on starch gel for PGM,EsD, EAP, and Glo-1 on whole fresh blood. They are now working satisfactorily. Haptoglobin is still not showing the sensitivity here that is routinely obtained by our consultants in their own laboratories. It appears to be a staining problem as opposed to separation. Variations in chemicals used for the staining solution are being investigated as a possible source of the problem.

Equipment has been ordered from Shandon to run Gc as a two-dimensional separation on agarose gel. It is now expected that all six systems of group 1 will be optimized for comparative testing about the 1st of May although methodologies for glyoxylase, EsD, and Gc on cellulose acetate require improvement.

Nanophore Status

The defective cooling plate has been reworked and received at the laboratory. It looks satisfactory and will permit runs to be made with gel plates requiring cooling. Results thusfar with cellulose acetate for PGM, EAP, AK, and other routinely-tested systems appear as good or better than those using the Microzone.

Work Planned for April

Major emphasis will be placed on optimizing the methodologies for analyses of aged samples. A new batch of four-week old samples will be available each week. This will permit a rapid iteration during optimization of techniques. The Nanophore unit will be operated with starch and acrylamide gels in addition to testing with cellulose acetate. Also, cellogel has been ordered and will be tested as part of the substrate selection process.

Cost Status

Technical accomplishment is consistent with expenditures. Please see the Monthly Cost Report for financial details.

Citations of Literature Regarding the Age of Bloodstains

E.A.P. "E.A.P. in Bloodstains" Wraxall & Emes, J. Forensic Sci. Soc. (1976) 16, 127-132. Page 130. "Discussion".

PGM - "The Determination of PGM Types in Bloodstains" Culliford. J. Forensic Sci. Soc. (1967) 7.3. 131-3. P. 131.

"Typing the Common PGM Variants Using IEF" Bank etal. J. Forensic Sci. Soc. (1976) 16, 115-20. P. 116. Results & Discussion.

HP "HP Types in Dried Bloodstains" Culliford & Wraxall. Native (1966). 211, 5051, P. 872-3.

GC "GC Types in Bloodstains" Wraxall. VIIth International Meeting of Forensic Science, Zurich 1976.

EsD Typing of EsD in Human Bloodstains Parkin and Adams, Medicine, Science, and Law 1975 Vol. 15, No. 2, P. 102-105.

GLOI GLOI in Bloodstains, Emes & Parkin. To be published - personal communication. "4-6 week old stains successfully typed".

MONTHLY PROGRESS REPORT

BLOODSTAIN ANALYSIS SYSTEM

Subcontract 67854

May 9, 1977

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Fourth Monthly Progress Report - Electrophoretic Bloodstain Analysis Program

This report reviews the work performed during the month of April 1977. The major tasks for this period were to continue optimizing the methodologies for selected systems using various substrates and electrophoresis cells. During this period, work was begun in evaluating aged stains on cloth and methods of extracting the bloodstains from cloth.

System Optimization

Working with fresh blood, 11 different systems had been optimized with various supporting media and using different cells. The status of this work is shown in TABLE I, fresh blood results. The difficulty which was experienced last month with haptoglobins using the gradipore unit was overcome and satisfactory results are now being obtained. Satisfactory results have been obtained with glyoxylase on starch gel with the Shandon cell. Additional work is being done for specific systems on various supporting media. For example, PGM is now being evaluated with starch gel on the nanophore unit. Glyoxylase is now being worked on the microzone with cellulose acetate. We are also continuing work to improve the results for GPT and GSR on cellulose acetate. At this time, the three main supporting media are cellulose acetate, starch, and acrylamide. Work is also being done with agarose and cellogel. Ultimately we would hope to reduce the supporting media to one or two types. The probability of discrimination based on the results of the available systems in fresh blood is 1 in 245. The major effort remaining for systems in fresh blood is GPT. With its discrimination index of .38, it would be capable of boosting the probability from 1 in 245 to almost 1 in 650. This would of course provide a greatly enhanced flexibility for combining systems for aged blood to achieve a good discrimination probability with a smaller number of systems and would be more practical to use in the crime laboratory.

Four-Week Aging Tests

In addition to stains on glass slides, during this month four-week-old stains from cloth were also analyzed. The majority of the samples are still from

blood from the OCJP program which contains ACD. However, fresh whole blood from individual donors has also been deposited on both slides and cloth and is being analyzed. The effect of the presence of ACD is being evaluated. The results are not yet complete, but so far indications are that ACD may act as an interferent in some cases while actually aiding the analysis in others. In any event it does not appear to be critical as far as the validity of our results are concerned. A summary of the work done so far on four-week old bloodstains from cloth is shown in TABLE II. The table shows that we have been successful in analyzing for nine different systems. To do this we have used three different supporting media and three different cells as a minimum. At this time six systems on one supporting media and one cell can be accomplished. With the nine systems, the probability of discrimination is 1 in 205. Although the major problem still lies ahead in combining the systems so that only three supporting media need be used to accomplish the 1 to 200 discrimination, at least it shows that at this time this discrimination probability is still possible to achieve.

Electrophoretograms from both the fresh and aged staining tests are being prepared for presentation at the next program review meeting. The next meeting is now scheduled for May 4th at the White Mountain Research Station, University of California, Berkeley.

Hardware Status

Most of the work reported this month was done using themicrozone and shandon cells. Work has started with both the nanophore and Beckman multizone cells. Although testing has been done with gradipore, pharmacia and visijar cells, no further work with these units is planned at this time.

The nanophore units which are being used are considered prototypes. Several minor revisions should be made to them to assure consistent performance. While these changes are desirable, it is not mandatory that they be made at this time. It is expected that further work will be done to evaluate the nanophore cell before any changes are made.

Work Planned for May

It is expected that during this month work will be completed in optimizing the methodologies for the selected isoenzymes. The optimization of the methodologies will still involve fresh blood. The effort on aged blood samples will continue with stains from both glass and cotton cloth. We will continue to investigate methods to obtain the maximum extraction of stains from cloth.

As a parallel effort to optimizing the analytical methodologies for individual systems, we will now begin to consider the combination of analyses to reduce the number of substrates and cells required.

Cost Status

Technical accomplishment is consistent with expenditures. Please see the Monthly Cost Report for financial details.

Table 1. Fresh Blood Results

		CAM		Starch		Acrylamide			Agarose			Other
D.I.	System	Microzone	Nanophore	Shandon	Nanophore	Gradipore	Microzone	Nanophore	Shandon	Microzone	Nanophore	Cellogel
0.47	PGM	C	C	C	IP							
0.35	EAP	C	C	C	C							
0.69	ESD	C	C	C								
0.38	GLO	IP		C								
0.39	Hp					C	C	C				
0.45	Gc	C	C	X	X				C①			
0.82	AK	C	C									
0.82	ADA	C	C									
0.91	PGD	C	C									
0.38	GPT	IP										
~0.95	Hb	C	C									
~0.92	G6PD	C	C									
~0.93	GSR	IP										

C = Complete
IP = In Progress
X = Excluded

① Antigen-Antibody Cross - Electrophoresis.

Table II. 4-Week-Old Bloodstains

		CAM		Starch		Acrylamide			Agarose			Other
D.I.	System	Microzone	Nanophore	Shandon	Nanophore	Gradipore	Microzone	Nanophore	Shandon	Microzone	Nanophore	Cellogel
0.47	PGM	C	C	C								
0.35	EAP	C	C	C								
0.69	ESD	C	C	C								
0.38	GLO			C								
0.39	Hp			X	X	C	C	IP				
0.45	Gc	C	IP	X	X				IP①			
0.82	AK	C		C								
0.82	ADA	IP		C								
0.91	PGD	X		X								
0.38	GPT											
~0.95	Hb	C										
~0.92	G6PD											
~0.93	GSR											

C = Complete
IP = In Progress
X = Excluded

① = Antigen-Antibody Cross - Electrophoresis.

MONTHLY PROGRESS REPORT

BLOODSTAIN ANALYSIS SYSTEM

Subcontract 67854

June 8, 1977

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Fifth Monthly Progress Report - Electrophoretic Bloodstain Analysis Program

This report reviews the work performed during the month of May 1977. The program is continuing in accordance with the established schedule. A program review meeting was held on May 4th at Berkeley. The laboratory work has continued on the optimization of methodologies and evaluation of alternate substrate materials. We began blind trials and work on multiple analyses on a single substrate.

System Optimization

The major effort in system optimization has been to refine resolution to obtain unambiguous readouts with aged stains on cloth. Operating parameters such as voltage, pH, buffer composition and concentration, and time have been varied in series experiments. It is obviously time-consuming to conduct a large number of experiments necessary to explore the effects of all the variables.

Most of the development work remaining to be done on single systems is with glyoxylase 1, Gc, GPT, and GSR on cellulose acetate. Glyoxylase 1 has not reached an acceptable level of resolution with CAM for fresh blood as yet. Gc is still somewhat erratic with stains on cloth. The methodology for Gc will be refined with repeated testing of the large number of samples. Work with GPT is also continuing but on a low level of effort. Analysis of GPT on aged stains is beyond the state-of-the-art. Many investigators have attempted this analysis and have not been successful. However, it is the intent of this program to advance the state-of-the-art. The potential increase in probability of discrimination from GPT is so great that we feel all ideas for its detection must be considered.

Only a low level of effort is being applied to GSR. It has a poor discrimination index in the general population but could have considerable utility in actual application. As it appears likely that it can be detected in aged stains, we have continued to work with it.

We expect to have our system defined by the end of June. That is, methodologies for all the selected isoenzymes would be completed. The next phase of effort in system development would then begin.

Substrates

Starch and cellulose acetate continue to be the most versatile substrates used. Acrylamide still seems to be indispensible to analyze for haptoglobins. Other substrates such as cellogel, agarose, silica gel, and a combination of agarose and acrylamide have been tested. Cellulose acetate membranes with variations in pore size have also been used. In some cases a specific advantage may be present but this is generally outweighed by other factors. For example, cellogel can hold more sample than cellulose acetate and might be more suited for low concentrations of certain isoenzymes such as GPT. But, as the membrane is thicker, it is more difficult to read and photograph with transmitted light.

It does not appear likely that a unique substrate will emerge that will accommodate all isoenzymes. We do hope that not more than two types of substrate will be necessary. Again, this problem should be resolved by the end of June.

Attempts to analyze for Gc on starch gel have not been successful. We have been able to detect Gc with both cellulose acetate and agarose gel. We are now trying to detect Gc on acrylamide. As acrylamide is only used for haptoglobins at this time, it is most desirable that at least one other component be detectable with it.

Cloth Stains

In comparing the detectability of aged stains on glass and cloth, our results so far indicate that they are about equal for the isoenzymes analyzed. The ease of obtaining samples for application to either starch gel or cellulose acetate is not significantly different. The volume of sample allowed seems to be ample for all systems which have been optimized.

Common Buffer

All individual systems have been optimized with a specific pH and buffer composition. In order to make multiple analyses on a single electrophoretic cell, a common buffer must be used. Some work has been done for multiple systems on both starch and CAM. So far testing has been with fresh blood only. Favorable results, that is showing feasibility, have been obtained with PGM, EsD, and GLO1 on starch. With CAM, four systems have been run. They are: Hb, AK, ADA, and 6PGD. It will be the primary effort of the next phase to finalize combinations so all selected enzymes can be analyzed on not more than three substrates and three cells.

Blind Trials

A series of tests are planned where coded aged samples will be analyzed with different substrates to determine which method provides the better analysis. Each phenotype will be read by a panel of four people and their individual readings compared with those from the original fresh blood. So far one trial has been made with EsD on starch and CAM. Equally good results were obtained with both substrates on the four-week old stains. In this particular test, an erroneous reading was obtained on fresh blood with cellulose acetate. The reasons for this have not been determined at this time.

A test with PGM was started but problems were encountered with both techniques. Troubleshooting the procedures indicated that one of the chemicals was suspect. The vendor was contacted to obtain a new lot. It is expected that all blind trials will be completed in June.

Hardware Status

It is expected that most of the work from now on will be conducted using the Beckman multizone cell which is a variation of the Shandon cell, and the nanophore cell.

One of the nanophore cells has been recalled by NASA so only one cell remains for our use.

In order to conduct the volume of tests necessary, we will continue to utilize the standard microzone and Shandon cells for this work. Final selection of the particular cell and modifications to it will evolve during the next phase of system development.

Work Planned for June

This coming month is one of the most significant ones in that data will become available to permit us to make several important decisions. The final selection of the specific isoenzymes that will be used for our total system, the optimum substrates and potential combinations should all be defined during this month. These selections and decisions will set the direction for the development of our system in the coming months.

Cost Status

Technical accomplishment is consistent with expenditures. Please see the Monthly Cost Report for financial details.

MONTHLY PROGRESS REPORT

BLOODSTAIN ANALYSIS SYSTEM

Subcontract 67854

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Sixth Monthly Progress Report - Electrophoretic Bloodstain Analysis Program

This report reviews the work performed during the month of June 1977. The program is continuing in accordance with the planned schedule. A program review meeting was held on June 21st at Berkeley. Dr. Grunbaum was on a trip out of the country this month at the invitation of the National Academy of Sciences and therefore unable to attend this meeting.

The main effort this month was in continuing system optimization and conducting blind trials to compare different techniques for the system analyses. A significant amount of time was spent troubleshooting the procedure for PGM. Established procedures which had worked previously for both starch gels and cellulose acetate membranes were followed in preparation for conducting a blind trial. However, neither method is yielding readable phenotypes. This problem area and details of other work are discussed in the following sections.

System Optimization

At the program review meeting, we listed 17 discriminators which had been considered for analyses for the four-week old stains. Out of these we have selected eight for our ultimate system. At this time only two are still undergoing development work.

One of these two is glyoxylase 1. A major effort was devoted to it with both starch gel and CAM. While the separation of GLO 1 is satisfactory on starch, the starch iodide detection method is very technique sensitive. A direct staining method has been sought but nothing as good as the iodide has been found yet. A two-step method using DCIP (dichloro indophenol) in an agarose overlay is promising but not yet reliable.

About 150 runs were made to find a way to separate GLO 1 on CAM. After screening buffer and pH combinations and successively selecting those that appeared to be optimum, the results are still not acceptable. A decision will be made shortly whether to continue this task or not.

The other discriminator which has received considerable attention has been Gc. It is being worked on agarose with antigen antibody crossed-electrophoresis, on acrylamide to try to combine it with haptoglobins, and on CAM. With fresh blood, Gc has been resolved quite satisfactorily on CAM. However, somewhat inconsistent patterns are obtained with aged stains. The biggest problem seems to be with sample deterioration and it is difficult to get enough Gc deposited on the membrane. A chloroform wash is necessary to get a clean separation but this also tends to cause some loss of sample. Therefore, the work has been directed towards better sample extraction and concentration. Also, a new staining technique to provide a better contrast is being worked on.

Gc has also been run on agarose using crossed-electrophoresis. The results of this method are good. However, this method is much more laborious than that with CAM. Since both use the same biochemistry and therefore inherently the same sensitivity, it again appears to be a matter of how much sample can be deposited on the membrane.

Other systems in which development work had been done during the month and which have now been dropped are GPT and GSR. With GPT many trials with both starch and CAM have failed to produce an acceptable procedure. Our experience has been similar to that reported in the literature. GPT is present in the blood in small quantities and undergoes rapid deterioration in stains. The degradation products obscure what little GPT is present so that satisfactory resolution cannot be obtained. Very little time has been spent on GSR. It has been decided to discontinue work on it as it contributes such a small amount to the discrimination factor in the general population.

Substrates

Most of the exploratory work on substrates has been with acrylamide and agarose. As acrylamide is necessary for Hp, it has been tested with PGM, EAP, EsD, GLO 1, and Gc also. Both gradient (4 to 25%) and homogeneous (10%) gels have been investigated. As PGM, EAP, EsD, and GLO 1 are separated primarily by charge, a gradient gel is not likely to be appropriate. The tests indicate the

activity of these systems is inhibited by polymerization products in the acrylamide. Only a weak response was found with EsD.

With homogeneous acrylamide, the results were not substantially better. Of the systems listed, only Gc appears to have some promise. With a 10% gel, Gc tends to separate rapidly and go off the end of the plate. With a 5 to 25% gradient, Gc packs into narrow bands. Perhaps somewhere in the 10 to 14% range, separation of both haptoglobins and Gc can be obtained.

Agarose has been used successfully for Gc as mentioned above. With other discriminators, it does not seem to give as good results as either starch or CAM. There seems to be some inhibition of the enzymatic activity. Whether this is due to using rehydratable membranes that may have some aging problems has not been determined. It does not appear likely that agarose would be a substrate of choice for any of the systems under consideration.

Blind Trials

The results of blind trials on EsD and EAP were presented at the program review meeting. These tests were based on cloth stains with the smallest sample volumes estimated to be 1 microliter or less. The results showed an incidence of reading error of the phenotypes about equal for either starch gel or CAM with EsD but with EAP, significantly less error was obtained with starch. These trials were the first conducted. They were found to be quite a bit more time-consuming than expected. It had been planned to continue blind trials with PGM and GLO 1. However, the problem with PGM delayed its trial and only one mthod is still available for GLO 1.

The major purpose of the blind trials is to aid in the selection of the best substrate for each specific system. This should help in grouping the various isoenzymes for multiple analyses. The blind trials would also help in showing the incidence of errors in reading phenotypes by a group of people. The blind trial results are not the basis for a final decision as to how an phenotype will be determined. A tradeoff analysis of the completed trials will be made

and this will set the direction for the development of procedures for simultaneous analyses. It may be that compromises will be made where it is necessary to analyze for one system on a substrate which did not show up best on the blind trials. This will all be done in consonance with the objective of providing unambiguous discrimination with the simplest system.

Hardware Status

It was determined that there are not really two independent Nanophore systems. Rather, there are two cells with common supporting equipment. However, the hardware has been received back in the laboratory and is now available to support our tests. Although certain desirable changes have been identified which might make it easier for an inexperienced person to get the best results with the Nanophore prototype, it is believed that the existing hardware can be tested as is and yield valid results.

Problem Areas

While we have the usual problems which are incident to any development effort, we have an additional one which has consumed so much time that it should be specifically pointed out as a problem area. This is with the troubleshooting of the PGM procedure. For approximately six weeks, work has been going on to obtain satisfactory results with the PGM procedures on both starch and cellulose acetate. Historically, problems with PGM have been traced to the inactivity or to a decrease of activity in the G6PD. In this case the source for the G6PD was contacted. This is Sigma Chemicals and an assay on the lot we use was requested. The assay indicated the activity to be normal. However, fresh material was received and tested. At first it appeared that that was indeed the trouble as satisfactory results were obtained. However, immediately on retesting again a lack of activity showed up. Other sources of G6PD were also contacted and samples obtained and trials made. Again, results have been variable. The storage containers for the chemicals have been examined, the distilled water that was used was checked; nothing has been identified as being a positive source of the problem. This has been most disconcerting in that it has taken a considerable amount of laboratory personnel's time and has caused

a significant interference in conducting our blind trials. Generally this type of problem yields itself to a methodical attack on an ingredient basis to determine the source of the problem and once it's found it can be corrected. In this case, the variable results have continued to obscure the basic cause of the difficulty.

Work Planned for July

The major effort will be in finishing the development work on GLO 1 and Gc and completing the blind trials. The troubleshooting on PGM hopefully will be over shortly. While the blind trials for the other systems can proceed, it is planned to run them all as close together as possible on the same stain. Therefore, it is desirable to get PGM working as soon as possible.

MONTHLY PROGRESS REPORT

BLOODSTAIN ANALYSIS SYSTEM

Subcontract 67854

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Prepared for:

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Seventh Monthly Progress Report - Electrophoretic Bloodstain Analysis Program

This report reviews the work performed during the month of July 1977. The program is continuing in accordance with the planned schedule. The main effort this month was directed toward completing the blind trials. We also solved the activity problem on PGM and EsD so that the blind trials on these constituents could be completed. We also continued optimizing the methodologies for the other constituents. The details of the work performed are discussed in the following sections.

Svstem Optimization

The development of the methodologies for the selected constituents has reached levels where we feel we have reliable and repeatable procedures for them. This means the capability of identifying the constituents in four-week old blood stains necessary to provide a discrimination probability of 1 in 200 has been achieved. We are of course still looking at the constituents on an individual basis and we do not wish to imply that there is still not room for improvement in several of them.

Although not strictly development work, the problem of the activity of PGM required a considerable amount of time and we are happy to report that we are now obtaining consistently good results with PGM. The investigation of this problem reveals that the activity level of G6PD as received from the vendor can vary considerably. Further, on reconstituting the enzyme, its rate of deterioration can also vary. It was found that it is necessary to use freshly reconstituted solution to assure that satisfactory analyses will be made. When the activity of the G6PD is correct, then prepared substrate plates can be stored for several weeks. This simplifies the procedure for cellulose acetate. It was found that with EsD on cellulose acetate, improved results were obtained when using a PGM buffer at a higher concentration than that which had been used in the initial blind trials. The buffer concentration was changed from a 1 to 20 dilution to a 1 to 7 dilution of the tank buffer. This tended to reduce the spreading of the bands and provided better resolution.

The extraction technique for preparing solutions for Gc samples was revised and satisfactory separations have now been achieved on cellulose acetate. It was found that it was desirable to obtain the maximum concentration possible of the sample solution. This permits an adequate amount of Gc to be deposited on the membrane with fewer sample applications. Cellulose acetate still appears to be the most practical substrate for Gc. The success of the analysis is still dependent on whether the Gc has deteriorated beyond the point of recognition or not. Work is still proceeding on analyzing for Gc using the antigen-antibody crossed electrophoresis technique. This is being done both on agarose and cellulose acetate substrates. So far the results are encouraging but considerable amount of development work remains before this technique would be satisfactory. The agarose substrate requires considerably more technician skill than cellulose acetate. This work is primarily of significance for our simultaneous analyses where we would hope that both Gc and haptoglobins might be combined on a single substrate. We have found that improved results for haptoglobin on acrylamide can be achieved using chloroform to clean up the sample.

Substrates

We are still working primarily with starch, cellulose acetate, and acrylamide. Work is continuing with agarose. At this time we need the three first substrates. However, if we develop the crossed electrophoresis technique for Gc and haptoglobins on either agarose or cellulose acetate then we would be able to eliminate acrylamide. From a toxicity standpoint this would be desirable. It would also be desirable in that the procedure with acrylamide requires a lengthy electrophoresis time. Our efforts to expand the capabilities of acrylamide for constituents other than haptoglobins have not shown any great promise. We have tried other means of polymerization and have gotten improved activity. We used dimethyl amino propyl nitrile instead of TEMED with ammonium persulfate. For our multi-system analyses, it is obviously desirable to either increase the acrylamide capability or eliminate it completely. In acrylamide polymerized with riboflavin, which has no enzymatic toxicity, is another available alternative. This may be explored in the future.

Blind Trials

Each time a blood stain is analyzed, we are in essence conducting a blind trial. However, in our efforts to assure that each system has reached a satisfactory level of development and where it is possible that a choice of substrates might be available, a formal blind trial has been made. The first of these trials were conducted in June and the results presented at our June program review meeting. Using the criteria that 90% of the stains must be identified correctly, none of the results of the first blind trials were satisfactory. It was therefore decided to repeat the trials that were conducted in June and also to conduct one on PGM which at that earlier time was causing considerable difficulty. In making the sample analyses, four-week old stains were used. On each plate along with the aged samples, 3-whole blood standard were also run. It was agreed that if the variants of the standard sample were not readable then the run was considered to be void in that normal results were not being obtained and that something had gone wrong with the analysis. Detailed information on the blind trials is provided in TABLE 1. The panel of readers varied from four to six people. One of the ground rules was that an attempt would be made to call each variant. If the reader had a doubt about a specific variant, he would call it and a question mark was added. In an actual crime lab, of course, there would have been no reading taken of a doubtful variant. The results reported here cover PGM, EsD, and EAP, where a possible choice of substrates was available. It can be seen from the table that on all of these substrates the variants of all three constituents were identified with a high degree of accuracy. The blood stains used for the samples for the blind trials were all four weeks old or older. They generally included four to five samples of whole blood plus samples from the OCJP program containing ACD. Total number of stains was 15 to 16. On starch, there was a total of 176 readings made. Out of these none were called incorrectly and only one was questioned. On cellulose acetate, there was a total of 264 readings. Out of these there was a total of 4 called incorrectly and a total of 8 questioned. The 4 incorrect calls made on cellulose acetate were all by the same reader. Seven of the 8 questioned calls were also made by the same reader.

Table 1. Blind Trials

Systems Tested: EAP: ESD: PGM:

Number of Trials Each System:

	Starch Gel	CAM
EAP	2	2
ESD	2	3
PGM	1	1

System EAP

1. Age of Stains	4 Wks.	4 Wks.
2. Number of Stains	15	31
3. Number of Readers	4	4
4. Total Number of Readings	64	109
5. Number Correct	64	106
6. Number Incorrect*	0	3
7. Number Questioned	0	3
8. Variants Present	2	2

*One Reader

System ESD

1. Age of Stains	4 Wks.	4 Wks.
2. Number of Stains	16	15
3. Number of Readers	3	5
4. Total Number of Readings	48	75
5. Number Correct	48	75
6. Number Incorrect	0	0
7. Number Questioned	0	2
8. Variants Present	2	2

System PGM

1. Age of Stains	4 Wks.	4 Wks.
2. Number of Stains	16	15
3. Number of Readers	4	6
4. Total Number of Readings	64	80
5. Number Correct	64	79
6. Number Incorrect	0	1
7. Number Questioned	1	5
8. Variants Present	3	2

Hardware Status

It was intended to use the Nanophore cell to run blind trials with cellulose acetate membranes. However, the sample applicator used with the Nanophore needs to be revised in order to apply the sample in a controlled manner where more than one application must be made. At present the tips are not held sufficiently rigidly so that exact placement can be duplicated. Some trial runs were also made using starch gel on Nanophore. The starch gel requires a cooling plate. Presently the cooling plate for the Nanophore cell is an all-plastic unit. Further the gel plate was also made of plastic. It was found that there was an inadequate cooling capability with this arrangement. The plastic cooling plate was revised so that one surface was converted to aluminum so that improved cooling could be imparted to the plate holding the gel. This new cooling assembly has not been tested at this time.

In using the microzone cells, it has been noted that there is an apparent current leakage across the partition which divides the cell. This leakage tends to shunt the substrate and causes a change in the current flow during electrophoresis. This current leakage causes a degradation in the resolution of the cellulose acetate membrane. This problem can be partially overcome by applying silicone grease to the top of the barrier. The microzone cells will have to be reworked to correct this problem so that the best performance can be obtained. The cells are being returned to the manufacturer for corrective action.

The new Beckman multizone cell is expected to be available in the month of August. It is hoped that several units will be available for use on this program. So far the Nanophore and multizone cells appear to be the ones designed to most conveniently accommodate a variety of substrates.

Results of System Definition Phase

It was the intent of this phase of our effort to select blood constituents which would give a probability of discrimination of at least 1 in 200, establish a methodology for identifying these constituents, and selecting the appropriate

substrates to be used. As a result of our efforts we have decided on eight constituents which the blind trials have demonstrated that we can identify accurately and which do provide the 1 in 200 discrimination capability. The results (Table 2) shows these constituents and the substrates which might be used for their analyses. In some instances our test results show that there is only one substrate which can be successfully used. In others it appears that we have a choice. Obviously the more options we have the more likely we are to be successful in performing simultaneous analyses with only three set-ups.

Work Planned for August

During this month work will commence on simultaneous analyses. Some work has been done with whole blood to check out some of the more obvious possible combinations. Work will continue to verify our capability on combinations using whole blood. For those combinations which appear to be workable with whole blood, we will then progress to using aged stains. We do not expect to do any further development work on individual constituents.

Table 2. Results of System Definition Constituents and Substrate Selection

Equivalent	Starch Gel	CAM	Acrylamide
PGM	GLO-I	Gc	H_p
EsD	EAP		
AK	ADA		

PR-2700-8

MONTHLY PROGRESS REPORT

BLOODSTAIN ANALYSIS SYSTEM

Subcontract 67854

September 8, 1977

Prepared for:

The Aerospace Corporation
Suite 4040, 955 L'Enfant Plaza, S.W.
Washington, D.C. 20024

BECKMAN

Advanced Technology Operations
Beckman Instruments, Inc.,
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Anaheim, California 92806

Seventh Monthly Progress Report - Electrophoretic Bloodstain Analysis Program

This report reviews the work performed during the month of August 1977. The program is continuing in accordance with the planned schedule. During this month, the system definition phase of the program was concluded. At a program review meeting on August 9th, the work done during this phase was reviewed and our conclusions presented. The remainder of the month was devoted to work on various groups of constituents for system development.

As the program review was documented with a hand-out and covered by minutes from the Aerospace Corporation, a detailed discussion of it does not seem necessary at this time.

System Development

A plan for the work to be done during system development was organized. The plan consists of three parts; listing of the major tasks involved, a logic network showing the sequence of events to be followed in developing individual group analyses, and a detailed work sheet showing tasks assigned and schedules. These tasks and the logic network were presented to Aerospace by a copy of a letter of August 18th addressed to Dr. Grunbaum.

The first groups that were established for development are: PGM, AKA, and EsD on cellulose acetate, and EAP, ADA and glyoxylase on starch. Haptoglobins and Gc were grouped together for both cellulose acetate and agarose substrates. Other groups considered but which were not specifically scheduled to be worked this month are PGM, EsD and GLO 1, and EAP, AK and ADA.

The initial efforts to make the group analyses of PGM, AK and EsD on CAM have pointed up a difficulty in getting a common buffer solution for PGM and EsD. PGM appears to require a 1 to 20 dilution of the buffer to get adequate separation using CAM whereas this dilution decreases the resolution of the bands for EsD. In order to achieve good definition for EsD, it appears necessary to maintain a higher buffer concentration on the order of 1 to 10 or 1 to 7. It seems like this group may be difficult to develop for cellulose acetate analysis.

So far the group analyses on starch have been made with PGM, EsD and glyoxylase. In this combination, after about two hours of electrophoresis, the EsD is stained and read. Then the PGM is stained and incubated, and finally the iodine solution applied for glyoxylase. Separation distances are adequate between PGM and GLO so there is no interference in the reaction. EsD, which reacts rapidly, is gotten out of the way and does not seem to interfere with the subsequent reactions.

In reviewing some of the earlier work done on rehydrated agarose gels, it appeared that separations were being obtained which were promising except for an apparent inhibition of the staining reactions. It was decided to investigate this further using freshly gelled agarose with the addition of a small amount of starch to provide the basis for the starch iodide reaction in staining glyoxylase. A combination of 1% agarose and 2% starch was made and the initial test was quite good. A simultaneous separation of PGM, EsD and glyoxylase showed good feasibility for this combination. At this time only a few experiments have been conducted and only a limited number of variations in pH and buffer concentration have been attempted. However, at this time the multiple analyses with this combination of agarose/starch appear to yield better results than earlier trials made with starch alone.

Another combination under consideration is EAP, AK, and ADA. Tests were also made on this group with agarose/starch. Of these, the ADA seems to be most difficult to separate from the initial tests. Not enough work has been done to tell what combination of buffer and pH will yield acceptable results.

Analyses for Gc have also been made on both CAM and agarose. The agarose substrate, with crossed electrophoresis, yields an easily readable result. However, it requires considerable skill to obtain good results. Major effort will be directed toward the combination of Hp and Gc on CAM. If this can be accomplished, it would be a significant breakthrough.

The investigation on cellulose acetate of the group PGM, AK, and EsD, has not shown a great deal of promise so far. The primary problem is the PGM and EsD incompatibility.

Confirmation Testing

Several blind trial tests have been made at various times. They were limited to a few constituents of major concern. In order to confirm the capability of making an unequivocal analysis on all the ingredients from a particular stain, it was decided to run a series of tests and analyze for all of the eight constituents at the same time.

We reviewed the library of aged stains to determine the variants which were represented in stains approximately 30 days old. We were able to find seven stains which gave a reasonably broad coverage of the various constituents; but some of these were only 14 days old, one was six weeks.

Using these seven stains, all eight constituents were determined. The stains were divided so that tests on starch and cellulose acetate were being conducted on an identical basis. For Gc, a comparative test was made using agarose and cellulose acetate. Also, for Hp runs were made with both step gradient and continuous gradient acrylamide gels.

TABLE I, attached, summarizes the results of the tests. It should be noted that EsD, PGM, and GLO I were run simultaneously on starch gel. On an individual run the results were definitely better. However, these results were very readable and show a strong promise for this combination.

Gc activity was evidenced in six out of the seven stains when run on agarose. On cellulose acetate, the first run attempted was not readable. This was attributed to an inadequate amount of sample being deposited on the membrane. A retest was made but the results were read by only one person as the other readers were out of the laboratory. In this test, four of the stains were called correctly.

Work Planned for Next Month

Several possible groupings for simultaneous analyses have been made. During this month tests will be directed toward establishing the most practical combinations. We will eliminate those combinations of constituents/substrates which the tests show to be least promising and concentrate our efforts on the rest.

PR-2700-8

-4-

TABLE I. RESULTS OF CONFIRMATION TESTS

Constituent	Number of Stains Analyzed	Number of Variants	Number of Readers/Readings	Number Questioned Cam	Number Questioned Starch	Number Correct Cam	Number Correct Starch	Number Incorrect Cam	Number Incorrect Starch
EsD(1)	7	3	4-28 CAM 4-27 Starch	1	0	28	24	0	3
PGM(1)	7	3	4-28	1	0	28	28	0	0
EAP	7	5	4-28	2	0	28	28	0	0
AK	7	1	4-28	0	0	28	28	0	0
ADA	7	2	4-28	1	0	27	28	1	0
GLO I(1)	7	2	4-25		3		24		1
Gc(2)	7	3	1-4 4-27 Agarose	0	3 (Agarose)	4	24	0	3
Hp	7	3	4-20 (Step Grad.) 4-22 (Cont. Grad.)	3 (Step Grad.)	2 (Cont. Grad.)	19	22	1	0
				8	8			2	7

NOTES: (1) EsD, PGM, GLO I run simultaneously on starch gel.

(2) Run twice on CAM. Only one reader present for retest.

We will also begin to determine our hardware requirements as they become more apparent from the group testing. Preparations will be made for a program review tentatively scheduled for the end of the month.

MONTHLY PROGRESS REPORT

BLOODSTAIN ANALYSIS SYSTEM

Subcontract 67854

October 7, 1977

Prepared for:

The Aerospace Corporation
Suite 4040, 955 L'Enfant Plaza, S.W.
Washington, D.C. 20024

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Eighth Monthly Progress Report - Electrophoretic Bloodstain Analysis Program

This report reviews the work performed during the month of September 1977. The program is now in the System Development Phase and is continuing in accordance with the planned schedule. Along with the laboratory work, consideration is now being given to the feasibility test plan and selection of hardware. These subjects are discussed in the following sections.

System Development

In August several groups of constituents were selected for development. One of these groups, PGM, AK, and EsD, was tried on cellulose acetate. The buffer requirements for PGM and EsD appeared to be too divergent to resolve in the limited time available so this work was stopped.

In view of this, it seemed prudent to concentrate our efforts for the other two possible major groups of PGM, EsD, GLO I, and EAP, AK, ADA on the starch/agarose substrates. On September 6th, a letter recommending this direction of effort was sent to Dr. Grunbaum. For the two serum proteins, Hp and Gc, work was to continue with several substrates. Dr. Grunbaum has assigned a technician to work full time with Beckman's consultant in support of our recommendation. During this month the group PGM, EsD, and GLO has been improved considerably. It is expected that this system will be finalized by mid-October. A 1% agarose gel with 2% starch to stain GLO is being used for this group. The other main group of three constituents, EAP, AK, ADA, is showing excellent results for EAP and AK. ADA is showing a loss of activity and may require lower voltage to reduce the heat generated. It may require overnight (16 hours) electrophoresis. We are confident that it will be a workable combination. This group is being run on starch with the expectation that it will be transferred to the agarose/starch combination.

There are two areas of concern with these combinations. One is the possibility that with serial separations the staining overlays may be improperly placed and mask some of the bands. The other, which is a contributing factor to the first, is variations in the agarose electroendosmosis from batch to batch and various suppliers. These problems can be controlled by using a parallel separation. The same stain must be applied side by side and the constituents are

isolated for staining. Depending on the number of standards used, this tends to limit the number of stains that can be handled on one plate with this approach but it precludes misapplying overlays. As the same basic methodology is used in either case, we can wait to see if the serial separation can be done repeatedly without interference. Our major technical problem is in combining Hp and Gc on one substrate.

We have tried to detect both proteins by antigen-antibody crossed electrophoresis (AACE). However, while the separation of Gc is excellent, the Hp phenotypes (due to very little charge differences between them) do not separate very well. There is also a certain amount of manual dexterity required when using this system.

Another approach now being tested is to separate the proteins on acrylamide, detecting the Hp with o-tolidine and the Gc by immuno-fixation. There are many combinations of buffers and acrylamide concentration and so it may be some time before the right combination is found.

It is possible to phenotype both Gc and Hp on acrylamide using serum samples but on stains there is an excess of Hb which tends to mask the banding. This approach is preferable to AACE if it can be worked out. In either case, the problems to be solved are:

1. Using AACE there is an interaction between the two antisera. Antisera from different manufacturers can be tried.

2. Finding the right combination of buffer and acrylamide concentration which will enable both Hp and Gc to be separated.

3. The excess Hb which occurs in bloodstains causes serious problems in Hp typing.

The group has promise but it requires a great deal of work before a decision can be made. However, if this combination does not work then individual systems can be used, but it would require an extra setup.

As a result of work being done on other programs at the Berkeley White Mountain Research Laboratory, it has been reported to us that some significant improvements have been made in the methodology for typing Hp and GLO-I. For Hp, the separation is done in the following manner.

Using a Nanophor type and size gel plate, 6% acrylamide gels (non gradient) are prepared for use the same day or on subsequent days. The gel thickness is 1 mm. At first blood samples were placed with a fine capillary pipet into slots that had been cut in the gel. Later, it was found possible to inject 8 samples simultaneously into gel by means of a Beckman Instruments, Inc., 8-sample plastic applicator. This sample application method is adequate but has not been optimized. The present plate will accommodate 12 samples.

In a number of experiments, a range from no cooling at all to cooling at about 5°C was tried. The best separations are obtained by cooling with tap water while electrophoresing for 45 to 60 minutes. It was also found, as expected, that staining time is considerably shortened. This is because of the ready penetration of the o-dianisidine stain into the 1 mm gel, as compared to the rate of penetration into the 3 mm gel which is used with the Beckman Microzone system. The longer staining time is required when using the Beckman system because the specimen itself runs in the geometrical center of the gel and is insulated with about 1 mm clear gel on each side. Also, because the o-dianisidine is not readily soluble in aqueous media, the amount of stain penetrating through the gel becomes smaller as it reaches the center of the gel. The total analysis time for Hp has now been reduced to approximately 90 to 120 minutes, as compared to the 16 hours needed for the usual step gradient method. Immunofixation on the thin acrylamide gels for Hp was also shown to be successful.

It is also indicated that a 6% acrylamide gel in a nanophor type and size gel plate is satisfactory for the separation and typing of the three common variants of GLO-I. Both fresh blood and bloodstains have been used. A few minor technical problems need to be worked out in placing the sample into the gel. This can be done with improved gel cutting sample application tips. As with Hp, this plate will also carry 12 samples.

This work offers a back-up approach for the entire group of analyses. This would utilize acrylamide for GLO-I and Hp and cellulose acetate for the six other constituents. Such an approach is outlined in the table below. With a single cell the analytical time limits could be met but five substrate plates would be required. No recommendation for pursuing this work on our program is being made at this time.

Order of Analysis	Substrate	Hands On	Elapsed Time
Sample Preparation	CAM	15 min.	15 min.
PGM	"	15 "	75 "
EsD	"	15 "	60 "
Ak-ADA-EAP (simultaneous)	"	25 "	60 "
GLO-I	Acrylamide gel	30 "	150 "
Hp-Gc (simultaneous)	"	35 "	120 "
	Total time	135 min. (2 h. 15 min.)	480 min. (8 h.)

Feasibility Test Plan

A preliminary plan is expected to be available by mid-October and submitted to Aerospace Corporation by November 1st. Some areas of concern are the referee methods and laboratories to be used to type the trial blood samples, the total number of stains for analysis, and how a mistrial for a particular stain will be handled. It should be noted that 64 laboratory worker hours are available for the tests. It is estimated that a maximum of 35 to 40 stains could be typed in this time.

Equipment Selection

Nothing has been finalized as yet in equipment selection. A choice between the smaller sized cells such as the microzone and the larger ones such as Shandon may be dictated by the size of gel plate needed. If a large cell is needed, the Beckman Multicell may offer the most flexibility in operation and lowest cost.

Work Planned for the Coming Month

A program review is scheduled for October 3rd. Our plans for completing System Development, the feasibility test plan, and hardware selection will be discussed at that time.

We are now rapidly approaching the time for final decisions in all of these areas. As of now we are still directing our efforts to fully comply with the statement of work. One possible compromise is that four substrate plates may be required instead of three. This has been identified as our major technical problem.

MONTHLY PROGRESS REPORT

BLOODSTAIN ANALYSIS SYSTEM

Subcontract 67854

November 10, 1977

Prepared for:

The Aerospace Corporation
Suite 4040, 955 L'Enfant Plaza, S.W.
Washington, D.C. 20024

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Advanced Technology Operations
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Tenth Monthly Progress Report - Electrophoretic Bloodstain Analysis Program

This report reviews the work performed during the month of October 1977. On October 3rd, a program review meeting was held. It was pointed out at the meeting that there is a difference of opinion about the methodologies to develop which would best serve the crime laboratories. The alternative approaches were discussed.

The alternatives are basically the use of gel plates versus cellulose acetate membranes for substrates. It has been stated in reports that the available starch gel methodology has not been generally accepted by the crime laboratories in the United States. The use of cellulose acetate membranes offered a possibility of reducing the skill level requirements for analyzing blood stains and also the time of analyses. However, the data from tests on this program shows the time requirements for technician effort is about the same with either substrate. Further, and most important, are the results which show that group analyses are most likely to be made successfully with the gel substrate. Therefore, based on the test results to date, it was decided to concentrate development effort on the gel plate methodology. This approach has shown the greatest potential to meet the requirements of the statement of work.

As a result of the decision to concentrate effort in this direction, it was decided that manpower could be more effectively utilized with laboratory facilities located at Beckman Instruments Advanced Technology Operation. Therefore, action to terminate work at UCB was initiated. On November 11th, the work under UCB cognizance will terminate. Arrangements are now being made to transfer the necessary personnel and hardware to the Beckman facility in Anaheim.

Technical progress made during October is discussed in the following sections.

System Development

Work is continuing with the three groups of constituents as identified at the program review meeting.

Group I

In this group, PGM, EsD and GLO are being separated on a 1% agarose, 2% starch gel plate. Variations of the relative concentrations of agarose and starch have been explored. This combination gives the best results.

Phenotyping of PGM and EsD is about equal to individual analyses. Results with GLO have been good at times but repeatability is poor. The GLO reaction mixture has been checked and does not seem to be a cause of difficulty. The buffer solution has not been completely checked out yet and this may be part of the problem. It was found that the bridge sponges need to be washed thoroughly or replaced when changing from one buffer to another.

The major problem with GLO has been with older stains (30 days). Comparative tests with individual analyses are now being made. It may be that the group analysis for GLO is actually doing as well as on an individual basis.

Group II

This group, EAP, ADA and AK, has been separated on 10% starch gels. Efforts to convert to 1% agarose/2% starch have not been successful.

In the past month the major problem had been with ADA. This has been much improved. Stains aged 4 to 5 weeks have been phenotyped. AK had been a problem in that it was not moving off the origin. This too has been corrected but there is still a problem with AK. The bands are being obscured by a smear that covers a fairly large area. It looks like the reaction stain has diffused. It may be that the reducing agent is too strong. The buffer concentration has been changed from 1 to 100 to 1 to 40. At the higher concentration, the pH jumped from 5.5 to 6.2 which was not expected. It may be that the problem with AK is related to the higher pH. The pH was adjusted back down to 5.5 for electrophoresing. So far, the EAP continues to come out very well.

Group III

The starch/agarose gel was also tried with this group, Hp and Gc, but did not work. Acrylamide still seems to be the best substrate. Results of tests this month have been encouraging in that some times both Hp and Gc have been readable on 30-day old stains. This indicates that it should be possible to separate these as a group. Generally one will come out but not the other.

Whether good results are gotten with Hp or Gc seems to be related to the extraction technique used for a particular run. It may be that the extraction procedure has denatured the proteins. Generally chloroform is used to reduce interference from Hb. While this does control the hemoglobins, it also deteriorates the Hp and Gc results. Perhaps a different solvent can be found to overcome this problem.

Crime Laboratory Test Plan

A draft copy of this plan was forwarded to Aerospace Corporation on October 24th for review and comments. This plan was not actually scheduled for submission until December 1st.

Feasibility Test Plan

A draft of this plan is now being prepared. It will be forwarded to Aerospace Corporation the first of November for review and comments. The plan is to be available in its final form on December 1st.

Hardware Selection

The need for gel plates large enough to accommodate separation distances of 6 to 7 inches precludes the general use of the microzone or nanophore type cells. The Beckman Multizone can be used with either gel or membrane substrates. We plan to use this hardware for the feasibility demonstration tests. This type cell will be commercially available from Beckman and a similar cell is available from Shandon.

Work Planned for Next Month

The need to establish a new laboratory set-up will require a major portion of time next month. An inventory of equipment and supplies will be made so procurement can be initiated for necessary items. Physical laboratory facilities must be arranged with adequate bench space and utilities. This can be done within the existing ATO building.

As time permits, development work will continue to finalize the methodologies. It is not expected that this work will be completed in time to conduct the feasibility tests by December 10th as scheduled. A delay of one month is projected now and this will be reviewed as soon as the transition of personnel and the laboratory has been completed.

APPENDIX

SECTION D

THE AEROSPACE CORPORATION

Suite 4040, 955 L'Enfant Plaza, S.W., Washington, D.C. 20024, Telephone: (202) 484-5500

3330-GNR-77-020
2 February 1977

Mr. Laird Morgan
Beckman Instruments, Inc.
Advanced Technology Operations
1630 S. State College Blvd.
Anaheim, California 92806

Dear Laird:

Please find enclosed the minutes of the 18 January 1977 Program Review. If you have any questions or if I can be of assistance, please contact me.

Very truly yours,

Gerald N. Roberts
Program Manager
Law Enforcement and
Telecommunications Division

GNR:jaw

Enclosure: As Stated

cc:
J. Bordeaux
B. Grunbaum
J. Walsh

BLOODSTAIN ANALYSIS SYSTEM

MINUTES OF MEETING WITH BECKMAN/BERKELEY

18 January 1977

The meeting was called to order at 9:20 a.m., Pacific Standard Time, at Beckman's Advanced Technology Operations in Anaheim, California.

Attendees:

The Aerospace Corporation

G. Denault
S. Derda
P. Jones
G. Roberts

Beckman Instruments

J. Bordeaux
J. Walsh
L. Morgan

University of California (Berkeley)

B. Grunbaum

PROGRAM PLAN

The meeting was opened by Mr. Bordeaux, Beckman Program Manager, who presented the attached PERT chart of the program and the master program schedule. After full discussion of these items, Mr. Bordeaux stated that Beckman would like to have the schedule changed for the procurement or modification of equipment needed for the crime laboratory testing. Work on the equipment was scheduled to commence after Aerospace notified Beckman of an acceptable feasibility demonstration test. Beckman would like approval earlier, if possible, during the feasibility demonstration test. Aerospace informed Beckman that approval to begin work on the equipment will be furnished to them in writing as soon after the feasibility demonstration test as possible.

Ms. Denault of The Aerospace Corporation laboratories suggested that the use of Nanophore might shorten the proposed schedule. Mr. Bordeaux did not agree and felt that, rather than allowing for more samples, the experimental apparatus would only enlarge the selection of possible substrates. Mr. Roberts, Aerospace Program Manager, disagreed and responded that the program required work with, and testing of, all substrates available, even if there was no Nanophore. Beckman agreed.

SUPPORT PERSONNEL

Mr. Bordeaux stated that Dr. Grunbaum, University of California at Berkeley, Principal Investigator, had dedicated space for the program's use at his laboratory. Dr. Grunbaum then stated that by 1 March 1977, the program would have two full-time employees, two part-time technicians, and himself as time allows.

Mr. Bordeaux stated that the program should have no trouble in obtaining the necessary materials, unless the items needed are out of the ordinary or exotic. Whatever was needed, though, would be made available.

Dr. Grunbaum discussed the training and background of the two technicians, both of whom are working full-time under a Law Enforcement Assistance Administration (LEAA) supported grant from the California Office of Criminal Justice Planning. Both of the individuals are forensic science graduates from Sacramento, California. Ms. Denault asked if the grant was going to run out soon. Dr. Grunbaum doubted that this would occur and was hopeful for an extension. Dr. Grunbaum stated that, even if the grant were terminated, our contract would still support one of the technicians full-time.

The status of Mr. Stolorow of the Michigan State Police Scientific Laboratory, and Mr. Wraxall of the Metropolitan Police Forensic Science Laboratory, London, was discussed. Mr. Stolorow is in the Berkeley area at the present time and is looking for housing. It is expected that he will be available to the program full-time by 1 February 1977. Mr. Wraxall

will not be available until 1 March 1977. The State of California Labor Department had to be informed that no one in the State was capable of performing the work that Mr. Wraxall is needed for. This notification will now be sent to the U.S. Department of State. Mr. Morgan, Beckman Instruments Contract Administrator, mentioned that the two consultant agreements had not yet been approved by The Aerospace Corporation. He was informed that this would be taken care of. (Note: Approval was received from LEAA on 1 February 1977.)

Dr. Grunbaum stated that Mr. Stolorow and Mr. Wraxall will be working under his direction. It was asked how disagreements in methodology between Dr. Grunbaum and the other members would be handled. Dr. Grunbaum stated that he intends to use the others for their ingenuity and will not dictate to them. If a concensus were not reached, the final report would reflect it. Mr. Walsh, Beckman Manager of Life Sciences, confirmed that both Mr. Stolorow and Mr. Wraxall knew of the arrangement and agreed to it.

FEASIBILITY DEMONSTRATION TEST

Mr. Roberts went through a list of agenda items; the first discussion concerned the feasibility demonstration test. It was stated that the subcontractor would have to be ready and able to test for all variants chosen by the subcontractor. It was assumed by Dr. Grunbaum and the Beckman representatives that the test samples would be on glass. Aerospace stated that the test samples would be dried bloodstained cloth. Aerospace further stated that, as far as crime laboratories are concerned, cloth is the only acceptable support. Most bloodstain evidence brought into crime laboratories is on cloth and the feasibility of the concept could not be proven on glass. It was also stated that crime laboratories must believe that the LEAA programs understand and appreciate their problems, and the use of glass was the logical baseline system on which their work should be performed.

A caucus was held between the Aerospace personnel to discuss the issue. The meeting lasted about 20 minutes, after which the program review continued. Ms. Derda, Aerospace Subcontracts Administrator, asked Beckman what changes in cost and schedule would be necessary in order to be able to fully develop the methodology for removing the bloodstains from cloth (no commitment by Aerospace was made). This was a Beckman and Berkeley action item and is due by 2 February 1977.

MONTHLY PROGRESS REPORT

The next item discussed was monthly progress reports. It was requested by Mr. Roberts that the monthly progress reports be delivered by the tenth of each month. Program reviews will normally be scheduled after the delivery of the most recent program report. It was further requested that the subcontractor redo the "estimate of consultant hours" and expand it to include a month-by-month breakdown per task item for each of the three researchers. Mr. Morgan agreed to furnish this within ten days.

NANOPHORE

The status of Nanophore was next discussed. Mr. Bordeaux informed Aerospace that the first prototype had tested well and many modifications were now planned. All of the modifications will probably be made at the same time, and several new models will be fabricated. Dr. Grunbaum displayed a number of photographs of the equipment. The Nanophore is the same length as the Microzone, but is 70 mm wide, rather than 55 mm wide as in the Microzone. The applicator tips are interchangeable so that each one can deliver a different quantity of blood, if desired. Dr. Grunbaum will attempt to have the photographs released by the National Aeronautics and Space Administration (NASA). It is Dr. Grunbaum's feeling that the Microzone could be modified to meet all of the Statement of Work requirements (Dr. Grunbaum has such modified instruments in his

laboratory), but he feels that the Nanophore will be able to do a significantly better job with greater ease. He also stated that GPT has been found to be unusable in dried stains and that glyoxylate may be a good replacement. Glyoxylate also has an excellent discrimination ability and basic work on this system's forensic applications are underway in London by Mr. Wraxall. In addition, Mr. Wraxall and Mr. Stolorow will work with equipment similar to that in their own laboratories (which will be purchased under this subcontract). The results of all testing, including Dr. Grunbaum's, will be intercompared to determine which investigator obtains the best results.

MISCELLANEOUS

Mr. Walsh was interested in determining if anticoagulants such as ACD could be used. It was felt that the use of these substances might lead to artificial and incorrect results.

Dr. Grunbaum asked if Aerospace had any suggestions on the methods to be used to collect blood samples. Aerospace felt that Berkeley was more involved with this than they were, and he was informed that he should use the methods he has already developed for the California grant.

Ms. Derda inquired as to where the funds were coming from to support the two part-time technicians. The new contract arrangement with Mr. Wraxall and Mr. Stolorow as Beckman consultants made approximately $23,000 available, enough to hire the technicians. Beckman was asked to determine if these individuals were necessary. If not, the excess funding might be put into other areas.

Mr. Roberts defined "technical direction" and stated that normal input at meetings was not, and should not be, construed as technical direction. Technical direction can only be given in writing from Aerospace contracts. If the subcontractor felt that technical direction was given, he should immediately contact Aerospace for written clarification.

ACTION ITEMS

1. Aerospace is to approve consultant agreements by 1 February 1977.
2. Dr. Grunbaum is to obtain release of photographs from NASA by 2 February 1977.
3. Response from the subcontractor on elution off of fabrics is required by 2 February 1977.
4. L. Morgan is to develop new man-hours and calendar charts by 27 January 1977.

AGREEMENTS

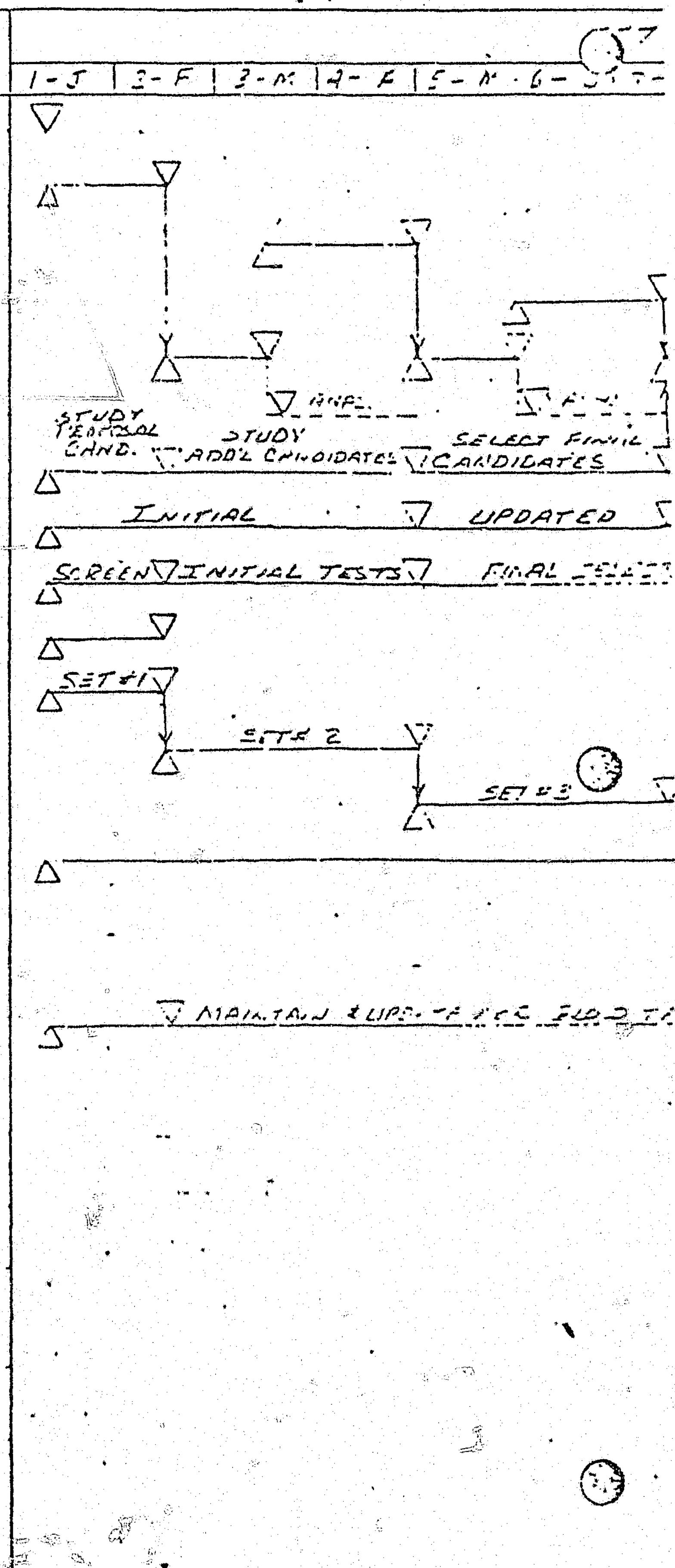
1. The Aerospace Corporation agrees to accelerating the schedule so that the crime laboratory demonstration test plan work will begin immediately after the feasibility demonstration test, rather than ten days prior to the crime laboratory testing.

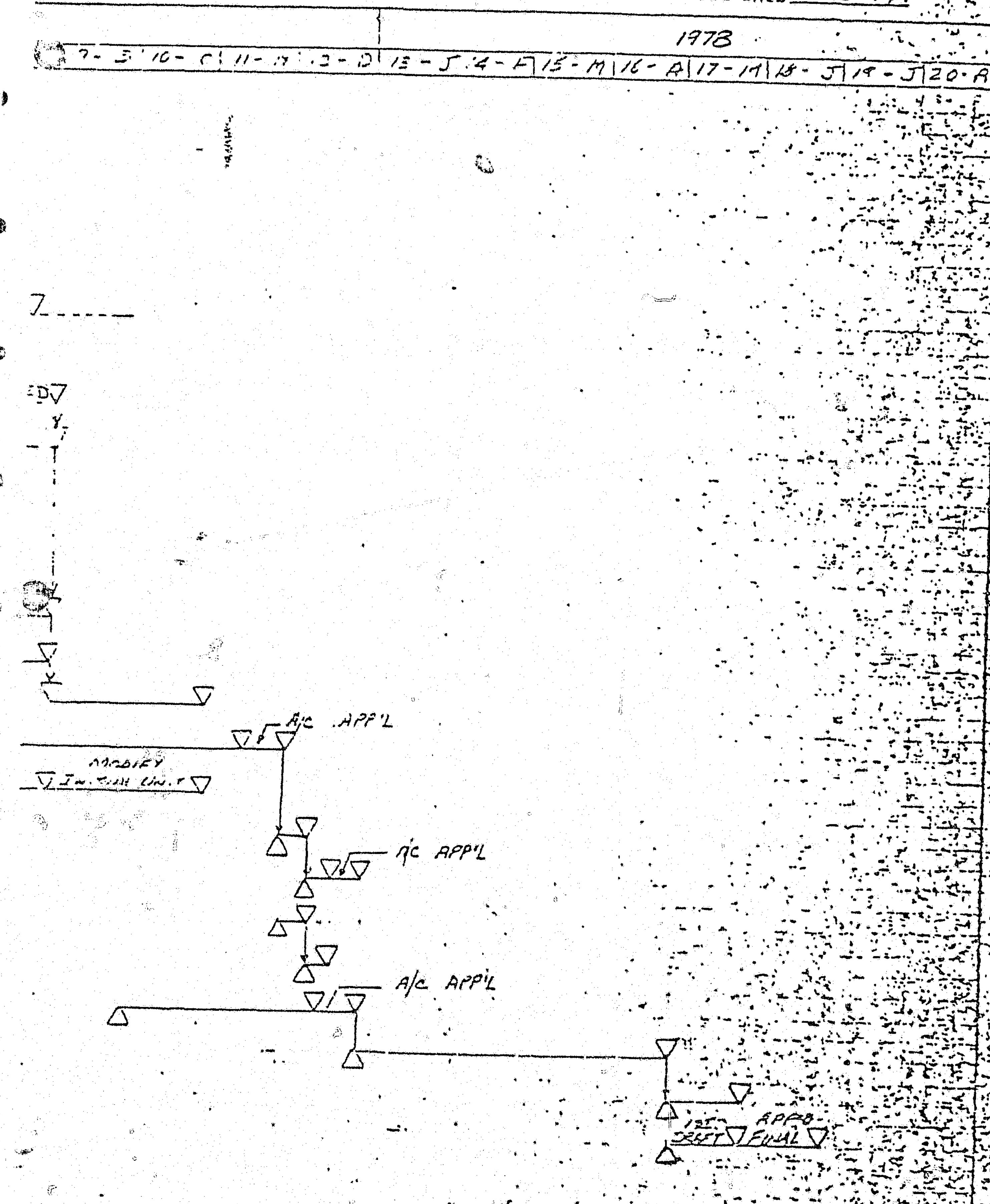
2. Equipment procurement/modification for five units is to occur upon direction of Aerospace as soon after the feasibility test as possible.

TITLE: BLOODSTAIN MASTER P... ...E PROGRAM ...N SCHEDULE

NO.
PAGE ____ OF ____ REV ____
ISSUE DATE 1-3-77

ITEM/TASK DESCRIPTION
PROGRAM GO-AHEAD
OBTAIN INITIAL BLOOD SAMPLES
ADD'L BLOOD SAMPLES
ADD'L BLOOD SAMPLES
BLOOD AGING (4 WEEKS)
BLOOD SAMPLE TESTING
SELECTION OF PROTEIN CANDIDATES
DISCRIMINATION PROBABILITY
SELECTION SUBSTRATES
ESTABLISH BLOOD STORAGE CONDITIONS
DEVEL. PRELIM. MEASUREMENT TECHNIQUE
DEVEL. ADD'L MEASUREMENT TECHNIQUES
FINALIZE MEASUREMENT TECHNIQUES
SYSTEM DEFINITION
SYSTEM DEVELOPMENT
DEVELOP FEAS. DEMO TEST PLAN
MODIFY MICROZONE FOR TESTS
FEASIBILITY DEMO TESTING
FEAS. DEMO REPORT APPROVED
PROCURE 5 MICROZONE SYSTEMS
MODIFY HARDWARE
DEVELOP CRIME LAB DEMO TEST PLAN
CRIME LAB DEMO TEST
CRIME LAB DEMO TEST REPORT
FINAL REPORT





PREP.
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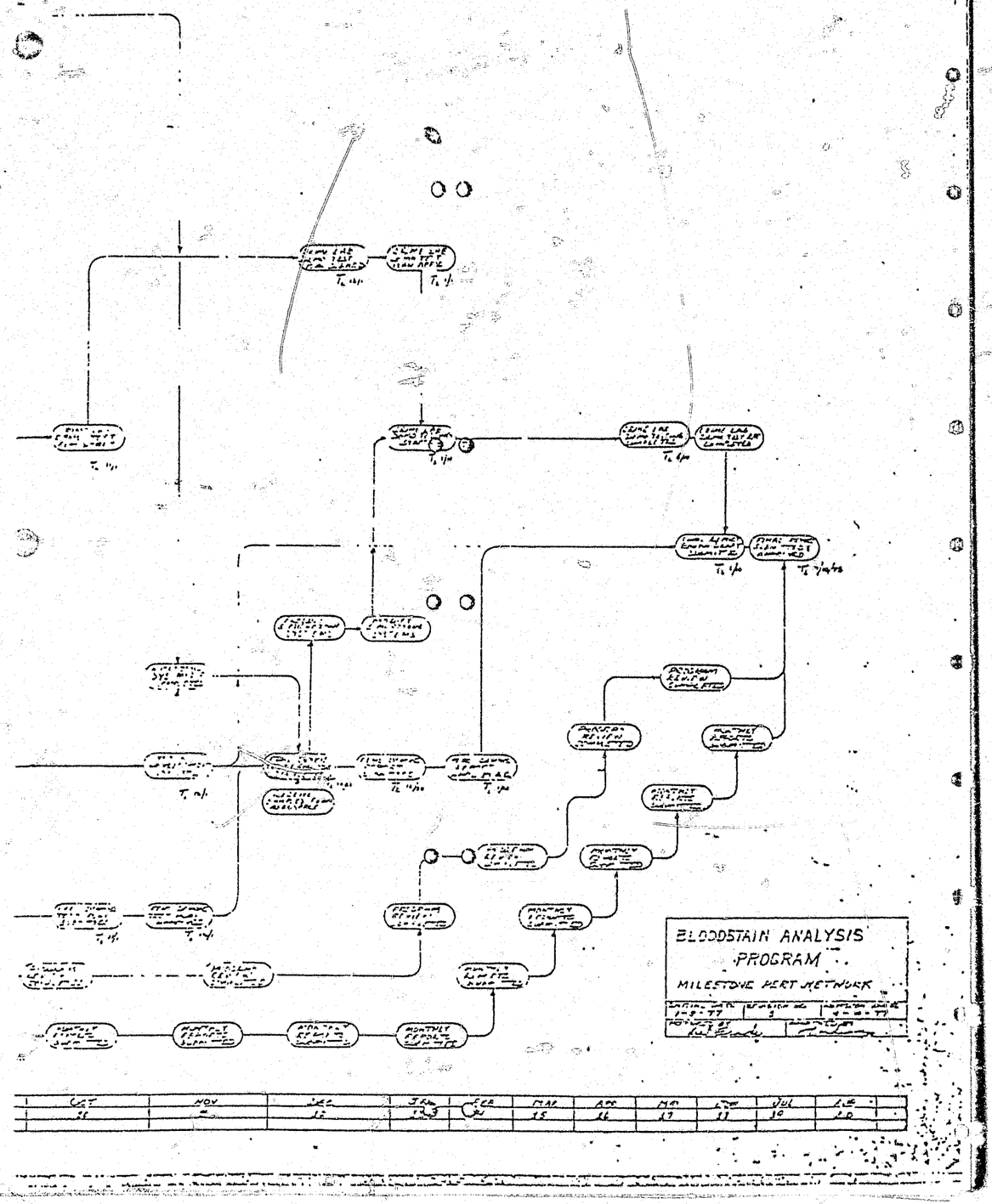
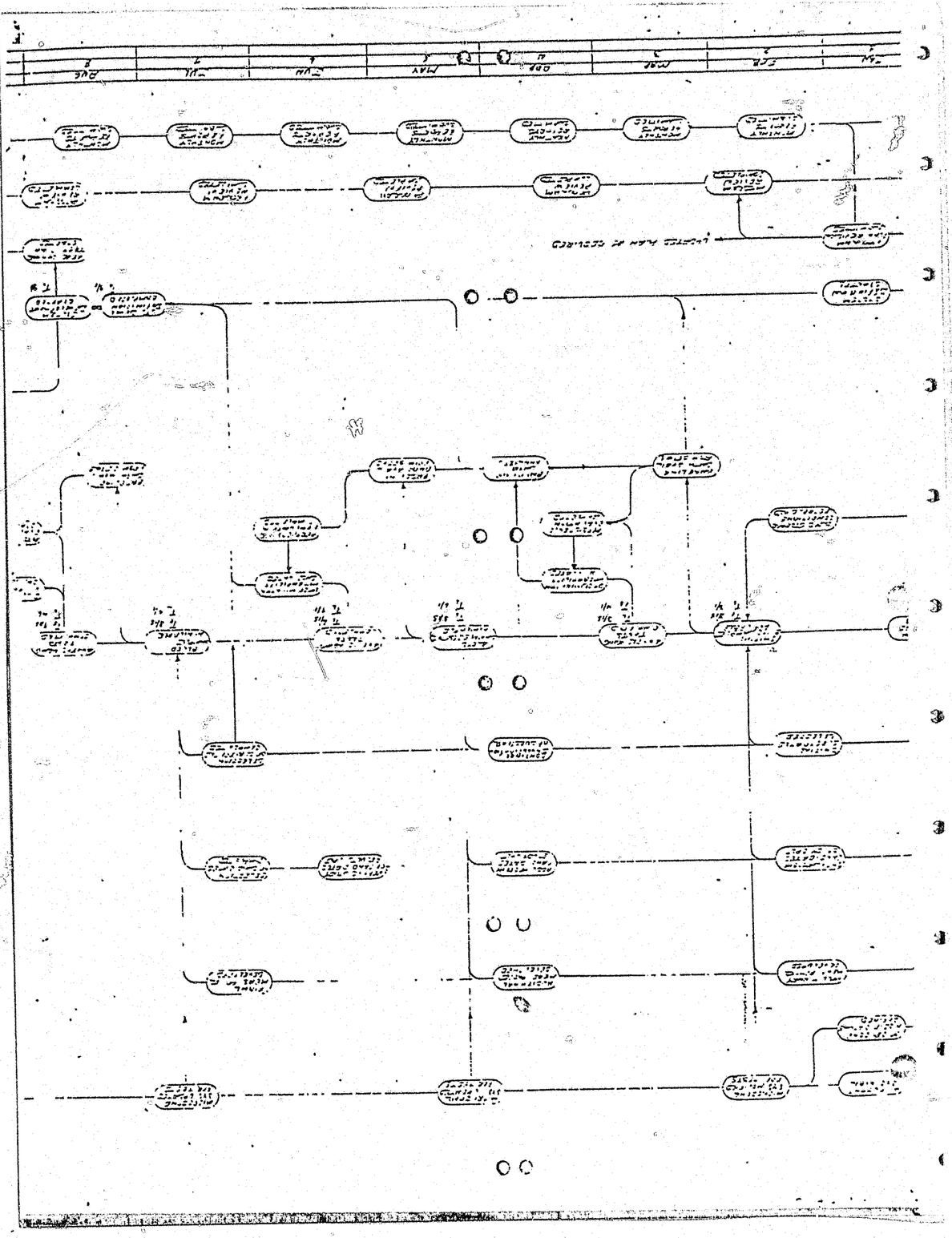

BLOODSTAIN ANALYSIS
PROGRAM
MILESTONE PERT NETWORK



THE AEROSPACE CORPORATION

Suite 4040, 955 L'Enfant Plaza, S.W., Washington, D.C. 20024, Telephone: (202) 484-5500

3330-GNR-77-023
23 March 1977

Mr. Jean Bordeaux
Beckman Instruments Inc.
Advanced Technology Operation
1630 S. State College Place
Anaheim, California 92806

Dear Jean:

Enclosed are the minutes of the second Electrophoresis Program Review held in Berkeley, California, on 15 March 1977.

If I can be of further assistence, or if you have any questions please contact me.

Very truly yours,

Gerald N. Roberts
Program Manager
Blood and Bloodstain Analysis

GNR:jaw

Enclosure as stated

cc: L. Morgan
W. Walsh
M. Stolorow
B. Wraxall
B. Grunbaum

MINUTES
OF THE PROGRAM REVIEW ON
ELECTROPHORESIS DEVELOPMENT
15 March 1977

The following agenda reflects the major items and topics considered at the Electrophoresis Program Review at Berkeley, California.

- Review of laboratory organization
- Examination of documentation available to eliminate unnecessary tasks
- Genetic marker testing
- Substrate testing
- Nanophore status
- Planned activities
- Problem areas
- Schedule
- Facility tour

The following persons were present at the meeting:

G. Roberts	The Aerospace Corporation
S. Derda	The Aerospace Corporation
G. Denault	The Aerospace Corporation
R. Kennel	The Aerospace Corporation
J. Bordeaux	Beckman Instruments
J. Walsh	Beckman Instruments
M. Stolorow	Beckman Instruments (Consultant)
B. Wraxall	Beckman Instruments (Consultant)
B. Grunbaum	University of California at Berkeley
G. Harmor	University of California at Berkeley
B. Del Re	University of California at Berkeley

TECHNICAL

The meeting was opened with an updated program control presentation by Mr. Bordeaux, Beckman Instruments Program Manager. The attached milestone PERT network was distributed. He explained that blood sampling and aging experiments were underway. The blood samples being used are not being selected for the variants which they possess, but rather those routinely arriving in Dr. Grumbaum's laboratory under the California Office of Criminal Justice Planning subcontract. Mr. Bordeaux further reported that all of the equipment specifically ordered for the use of starch gels and acrylamides have arrived and are operational.

Dr. Grunbaum, Principle Researcher, felt that one of the major accomplishments of the program was bringing internationally qualified persons to participate in the program. The general plan for whole blood analysis was then explained. Both of Beckman's consultants resident at Berkeley, Mr. Mark Stolorow and Mr. Brian Wraxall, will work on improving the analysis on the presently run starch gel and acrylamide methods while Dr. Grunbaum's two assistants, Mr. Del Re and Mr. Harmor, will utilize the cellulose acetate techniques. The methods of the researchers will be inter-compared and the best ones choses. Weekly meetings are held with all researchers present to compare results and outline the following week's work. It is planned that the decision on which methods are to be used will be reached by mutual agreement among the researchers. If mutual agreement is not achieved, the disagreement will be discussed and resolved at a meeting with The Aerospace Corporation with all program participants present.

Dr. Grunbaum also stated that eventually all of the researchers will learn to use and operate all electrophoretic equipment: starch, acrylamide, cellulose acetate, including Nanophore.

The topic of discussion turned to the genetic systems which will be tested. The Aerospace Corporation Statement of Work calls for the investigation of a minimum of fourteen (14) genetic systems. One of these, GPT, has not previously been run in this country. Preliminary results on fresh blood look promising, but since blood has a very low concentration of this substance, the method may not work on aged and dried blood. Another substance, glutathione reductase has a good potential, as seen in the early tests. Mr. Stolorow has been working on carbonic anhydrase and peptidase-A, but no decision has been made on these as of yet. Both of these systems have poor discrimination abilities which may limit their usefulness.

Dr. Grunbaum distributed a summary chart (attachment 2) compiled by Mr. Wraxall which listed all the likely genetic systems broken into three (3) groups, in order of priority to the program. The first group, composed of EAP, HP, PGM, GLO-I,Gc and EsD, could provide a cumulative discrimination of 1 out of 250 if isoelectric focusing were used for the PGM, or 1 out of 133 without the use of this technique. It was stated that the group I systems were reliable, persistant and afforded good discrimination.

The addition of group II systems, AK, ADA, 6PGD and GPT would increase the total number of genetic system to ten (10) and the cumulative discrimination from 133 to 215. It was felt by the researchers that the addition of all of these systems would probably not be worth the return of the increased discrimination. They also concluded that the group III systems, (PCE, UMPK, Hb, GR, G6PD, PEP-A, and CA II) will probably not be considered because of their poor discrimination, except for specific ethnic groups.

Dr. Grunbaum and Beckman's consultants have decided to concentrate their development efforts on cellulose acetate, starch gel and

acrylamide gel. They realize that all substrates listed in the Statement of Work must be completely tested unless documentation to prove lack of feasibility can be furnished to Aerospace.

The iterative process for determining the most promising genetic systems and electrophoretic substrates should be completed by 1 August 1977. This segment of the study is intended to optimize the separation and technique against all of the requirements and facets of the Statement of Work. When the task has been completed, the planning and preparation for the Feasibility Demonstration Test will begin.

A decision was reached on the use of the anti-coagulent ACD which is added to all the blood which Dr. Grunbaum receives under the California Criminal Justice program. The use of ACD will be permitted in the early development stages of this program, but before any genetic group is accepted as a definite component of the finished system that group will be completely tested with blood containing no perservative or anti-coagulent. It is similarly recognized that the blood samples used in the Feasibility Demonstration Test also will contain no additive.

The research approach outlined above will be continued through the next reporting period, in addition all of the analysts will work on "blind samples" which had previously been characterized.

INSTRUMENT TESTING

The development plan which will be followed in the next month calls for the testing of the modified Microzone, Nanophore, Gradipore, Shandon, and the Beckman Multi-zone (a new technique based on modifications to Shandon equipment). This will hopefully resolve not only the identification or the best genetic systems but the best equipment approaches also.

For the present time the Nanophore units will be used for cellulose acetate only since the temperature control plate has buckled and cannot be used. It is anticipated that a new or repaired plate will be obtained shortly.

HAZARDOUS MATERIALS

The discussion turned to the point of possibly toxic substances and their use in any development system. No substance presently banned by OSHA or other federal agencies is being used. Aerospace will be advised by the subcontractor about the use of any potentially hazardous material. Aerospace approval for the use of any hazardous substance is required, as documented in the Statement of Work.

TRAINING

There was also considerable discussion concerning the extent of the training necessary for criminalists to effectively use any system developed. The training is dependent on the individual criminalist, but two weeks does seem reasonable as a starting point. Not only is training documentation material planned, but also, possibly, direct training from the research team.

ACTION ITEMS AND AGREEMENTS

None.

Electrophoretic Bloodstain Analysis Programme

1st. Priority Grouping Systems

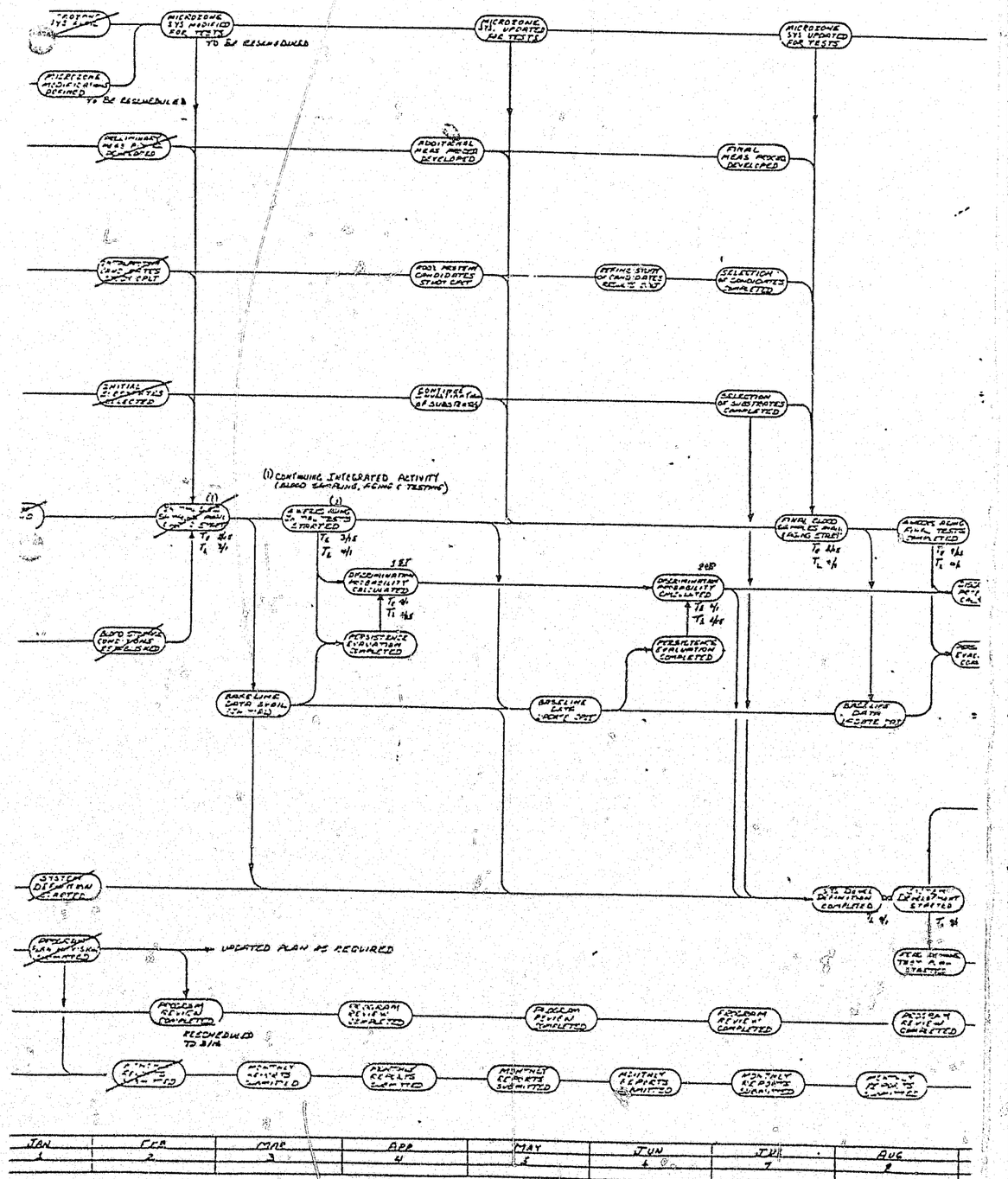
SYSTEM	DISC. PROB.	COMMENTS
EAP.	0.35	
Hp.	0.39	
PGM.	0.25(0.47)	IEF. should be used.
GLO I	0.38	
Gc.	0.45	Stains can be unstable.
Es.D	0.69	
Total	0.0040(0.0075) =	I in 250(I in 133)

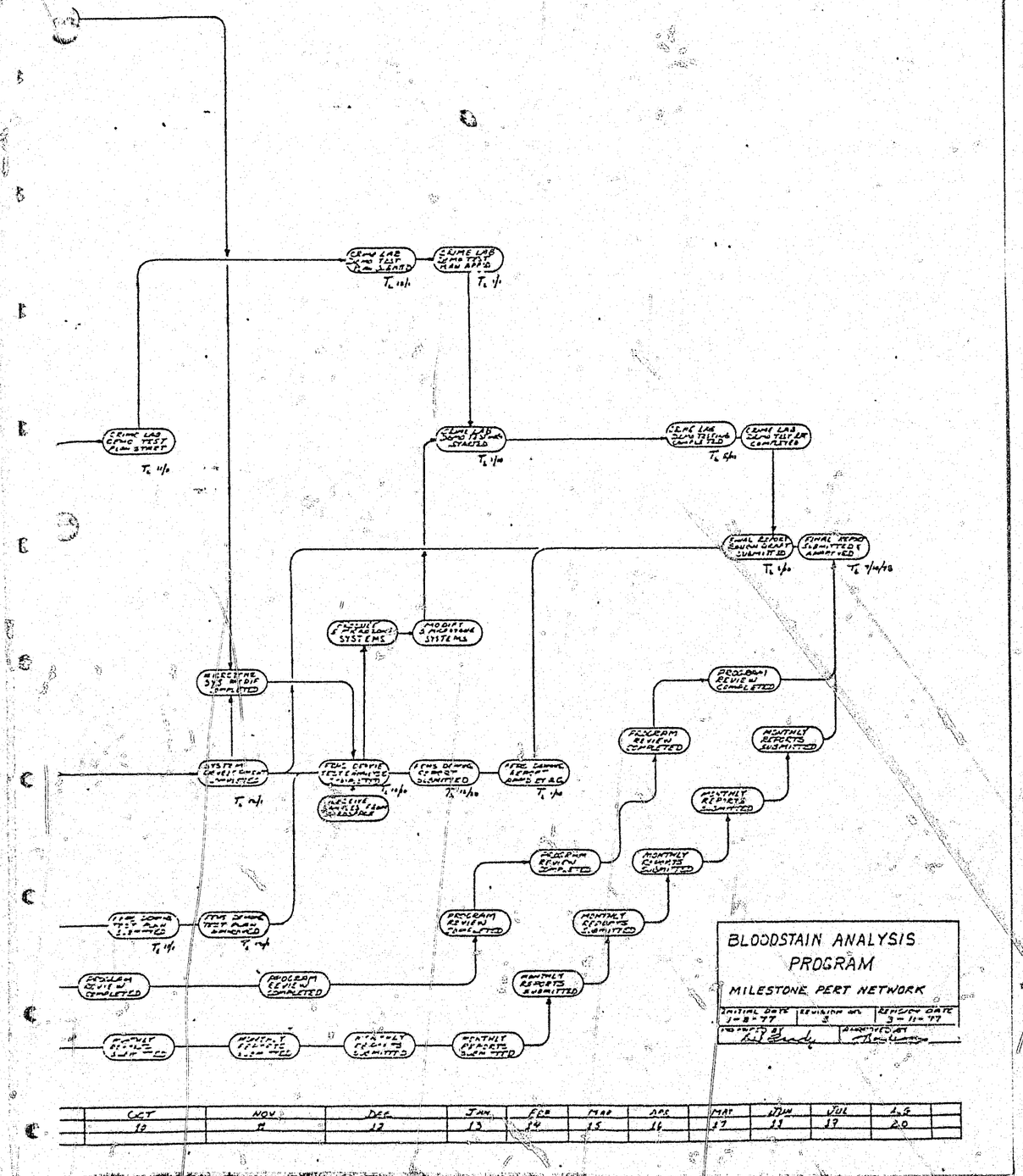
2nd. Priority Grouping Systems

AK.	0.82)	Could be completed on index
ADA.	0.82 ---)	one substrate at same time
6PGD.	0.9I	
GPT.	0.38	Unreliable. Stains are unstable.

Systems to be Rejected

PCE(EI&2)	0.82	Completely unreliable.
UMPK.	0.82	" "
Hb.	0.82(Blacks)	D.P. very low in combined population.
GR.	0.59(")	" " "
G6PD.	0.4I(")	" " "
Pep.A	0.75(")	" " "
CAII	0.75(")	" " "





THE AEROSPACE CORPORATION

Suite 4040, 955 L'Enfant Plaza, S.W., Washington, D.C. 20024. Telephone (202) 484-5500

3330-GNR-77-028
May 12, 1977

Mr. Jean Bordeaux
Beckman Instruments, Inc.
Advanced Technology Division
1630 South State College Place
Anaheim, California 92806

Dear Jean:

Enclosed are the minutes of the third Electrophoresis Development Program Review held at the White Mountain Research Station in Berkeley, California, on 4 May 1977.

If I can be of further assistance, or if you have any questions, please contact me.

Very truly yours,

Gerald N. Roberts
Program Manager
Blood and Bloodstain Analysis

GNR:ns

Enclosures (as stated)

cc:
L. Morgan
W. Walsh
M. Stolorow
B. Wraxall
B. Grunbaum

MINUTES OF PROGRAM REVIEW
ON ELECTROPHORESIS DEVELOPMENT
4 MAY 1977

The following is the agenda of the Electrophoresis Development Program Review held at the White Mountain Research Station, Berkeley, California.

I. STATUS OF ISOENZYME OPTIMIZATION

- A. Fresh Blood
- B. Aged Blood
- C. Samples Deposited on Cloth
- D. Cumulative Discrimination Calculation

II. EQUIPMENT OPTIMIZATION

- A. Nanophore
- B. Modified Microzone
- C. Other Systems

III. PROBLEM AREAS

- A. Hazardous and Potentially Hazardous Substances
- B. Others

IV. FUTURE PLANS

The following persons were present at the meeting:

The Aerospace Corporation	Beckman Instruments
G. Denault	J. Bordeaux
G. Roberts	M. Stolorow (Consultant)
R. Shaler	J. Walsh
	B. Wraxall (Consultant)

University of California at Berkely
B. Del Re
B. Grunbaum
G. Harmor

TECHNICAL

Mr. Bordeaux opened the meeting by distributing and discussing the attached updated PERT milestone network. After some discussion, Dr. Grunbaum commenced the technical presentation. Results to date indicated the EAP, EsD, PGM, Gc, and Hp can all be detected up to a minimum of four weeks in dried stains. In addition to glass, these stains were removed from pieces of cloth as small as 2 square millimeters. Stains have been extracted by a number of techniques, including:

- Simply soaking the cloth in a small amount of water, then placing the solution on the electrophoresis substate.
- Extracting the blood by primary rinsing, centrifuging and vortex mixing in a specially designed capillary chamber, then cleaning the extracted sample with chloroform. There is no indication that the sample size requirement of the Statement of Work will be any trouble to meet.

Some work on combining several systems on one cellulose acetate membrane has been undertaken. The four systems, Hb, AK, ADA, and G6PD, have all been run simultaneously on one piece of the substrate material. Work has been conducted on reducing the quantities of reagent needed to visualize the group-specific component, Gc. This work has progressed to the point where only nanogram quantities of the component are required. Other systems which also look hopeful are Bis-Albumin, GsR, EAP, Hp, and GPT.

The next presentation was made by Mr. Wraxall who described the joint work performed by Mr. Stolorow and him. The two attached charts describing which genetic systems have been tested on which substrates were distributed. Work on eight genetic systems (AK, ADA, PGM, GLO, EAP, EsD, Hp, and Gc) has been conducted on dried blood stains on cloth.

All were detectable in excess of the 4-week persistence requirement. No interpretation problems were caused by the addition of the anticoagulant ACD to the blood prior to staining, except in the Hp system where there was a problem in correctly interpreting phenotypes due to the addition of the ACD in the blood. Most of the work is being performed on starch, but other substrates such as acrylamide, agarose, and cellulose acetate are also being tested. Most of this work is being conducted without the need of extractions; in practice, one or two threads of cloth are soaked in the buffer solution and then inserted into the gel material. Some problem has been encountered in the Gc system which seems in a large extent to be dependent upon the sample history. This may cause practical problems for crime laboratories where samples are exposed to moist air and/or warm temperatures. Degradation of the Gc variants in these samples does occur.

Some work on running simultaneous samples on the same substrate have been conducted by these researchers; preliminary results indicate that EAP, GLO, EsD, and ADA could all be run on the same starch gel plate. Discrimination of 1 in 200 appears to be reasonable and obtainable. When all eight systems listed above are utilized, a cumulative discrimination of 1 in 196 is obtained. The addition of Hb increases this number to 1 in 205.

EQUIPMENT

Work has been underway on all of the off-the-shelf equipment, as well as the modified Microzones and Nanophore. Work on the Multizone is just beginning, and its potential usefulness is not known. In order to assure that the temperature control plate on the Nanophore is providing uniform temperature, a thermister will be implanted in the starch gel as a monitor.

PROBLEM AREAS

A list of seven substances which might be considered hazardous has been developed. These materials are:

- O-tolidine
- Cyanogum 41 gelling agent
- Leuco molachite green
- TEMED
- 2-Mercaptoethanol
- MTT
- PMS

All of these substances will be checked with the Occupational Safety and Health Administration to ascertain if any are regulated. This is an action item for Aerospace and is due by the next program review.

ACTION ITEMS

Aerospace is to ascertain the hazardous potential of the seven substances cited above. This is due by the next program review.

AGREEMENTS

None.

I. FRESH BLOOD

✓ COMPLETE
IP IN PROGRESS
X EXCLUDED

D.I.	SYSTEM	CAM: MICROZONE	CAM: NANOPHOR	STARCH: SHANDON	STARCH: NANOPHOR	ACRYLAMIDE: GRADIPORE	ACRYLAMIDE: MICROZONE	ACRYLAMIDE: NANOPHOR	AGAROSE: SHANDON	AGAROSE: MICROZONE	AGAROSE: NANOPHOR	OTHER: CELLOGEL
0.47	PGM	✓	✓	✓	IP							
0.35	EAP	✓	✓	✓	✓							
0.69	EsD	✓	✓	✓								
0.38	GLO	IP		✓								
0.39	Hp					✓	✓	✓				
0.45	Gc	✓	✓	X	X				✓①			
0.62	AK	✓	✓									
0.62	ADA	✓	✓									
0.91	PGD	✓	✓									
0.38	GPT	IP										
~0.95	Hb	✓	✓									
~0.92	G6PD	✓	✓									
~0.93	GSR	IP										

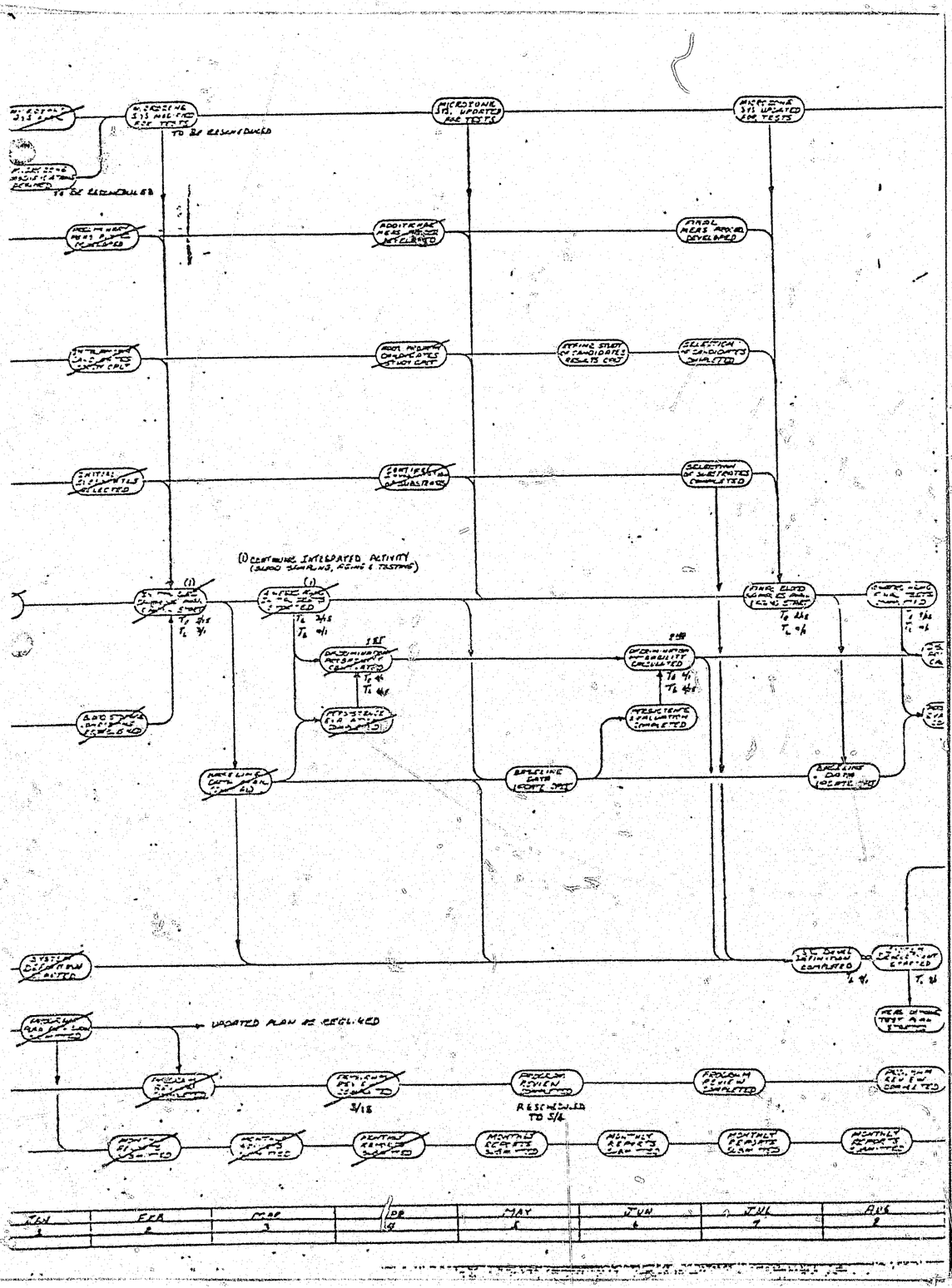
(1) (1)

II. 4-WEEK-OLD BLOODSTAINS

✓ COMPLETE
IP IN PROGRESS
X EXCLUDED

D.I.	SYSTEM	CAM		STARCH		ACRYLAMIDE			AGAROSE			OTHER	
		MICROZONE	MANDRAKE	STANDARD	MANDRAKE	GRADIENT	MICROZONE	MANDRAKE	STANDARD	MICROZONE	MANDRAKE		CELLOGEL
0.47	PGM	✓	✓	✓									
0.35	EAP	✓	✓	✓									
0.69	EsD	✓	✓	✓									
0.38	GLO			✓									
0.39	Hp			X	X	✓	✓	IP					
0.45	Gc	✓	IP	X	X				IP ①				
0.82	AK	✓		✓									
0.82	ADA	IP		✓									
0.91	PGD	X		X									
0.38	GPT												
~0.45	Hb	✓											
~0.92	G6PD												
~0.93	GSR												

① ( )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TO BE RESCHEDULED
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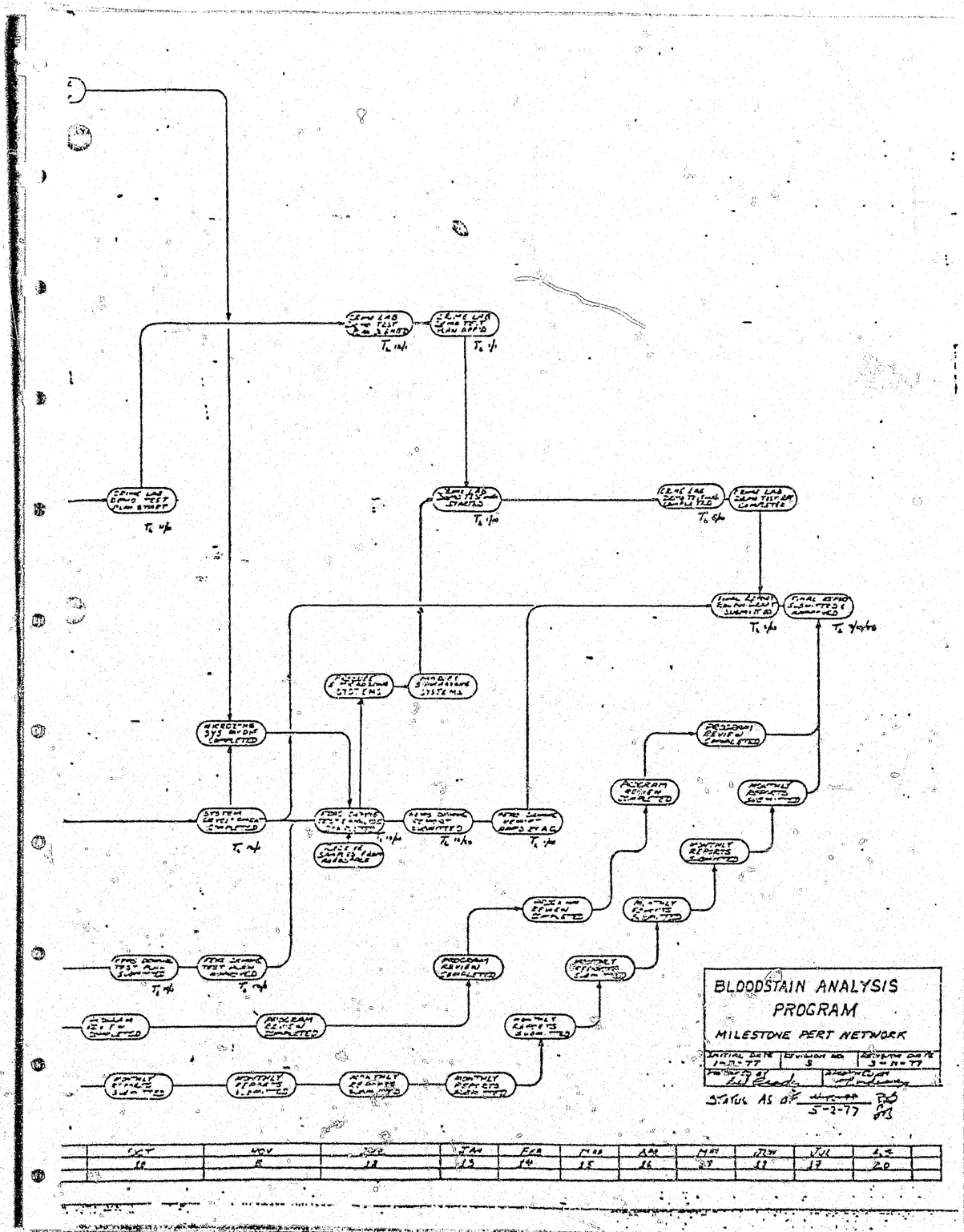
SRT
SRM
UPDATED PLAN AS REQUIRED
3/15
RESCHEDULED TO 5/4
JAN 3
FEB 6
MAR 3
APR 4
MAY 5
JUN 6
JUL 7
AUG 8


BLOODSTAIN ANALYSIS PROGRAM
MILESTONE PERT NETWORK
STATUS AS OF 5-2-77
OCT 10
NOV 11
DEC 12
JAN 13
FEB 14
MAR 15
APR 16
MAY 17
JUN 18
JUL 19
AUG 20

BLOODSTAIN ANALYSIS SYSTEM
MINUTES OF MEETING WITH BECKMAN/BERKELEY
20 JUNE 1977

The meeting was called to order at 1:15 P.M. (Pacific Standard Time) at the White Mountain Research Center, University of California.

Attendees:

The Aerospace Corporation

G. Denault
R. Kennel
S. Derda
G. Roberts
R. Shaler

Beckman Instruments

J. Bordeau
L. Morgan
M. Stolorow
J. Walsh
B. Wraxall

University of California Berkeley

B. Del Ray
G. Harmor

June Minutes, later replaced by "official" copy. See July 21 letter. BWE

Equipment Optimization

Mr. Bordeau discussed the progress regarding equipment optimization. The nanophore was not used during this period because of its removal from the laboratory by NASA. The reasons for the removal of the equipment was not known, and its return to the laboratory is expected within one month. The other items of equipment: the Multizone and Shandon units are being used extensively; the Gradipore apparatus has extreme limitations in these studies.

System (Isoenzyme) Optimization

Fresh Blood

Mr. Stolorow presented the results to date concerning the optimization of the isoenzyme systems.

1. A schematic was presented (Attachment 1) which illustrates the rationale being used in order to eliminate inefficient efforts regarding the use of the different substrates or equipment. The flow diagrams show that various substrates (acrylamide, starch or cellulose acetate membrane (CAM)) will be investigated with each system. The system which gives the best results with a particular substrate will then be studied with blind trial results, and detectability limits being used as selection criteria. These studies should then permit a decision to be made regarding the acceptability of one or more substrates for each system. Once the optimization for each system on a particular substrate (s) is determined, the appropriate item of equipment will be selected.
2. An update chart was presented (Attachment 2) which shows the progress regarding the optimization of each system on the different substrates. The chart illustrates that all Group I systems are still being evaluated. The following preliminary conclusions have been arrived at since the previous program review:

   A. PGM, EAP, EsD have been eliminated from acrylamide. This elimination is due to both theoretical considerations and experimental results. The results showed that acrylamide severely inhibits the enzyme activities of these systems.

   B. GLO has been tentatively eliminated on CAM because of poor resolution of the bands.

   C. The above systems are also being investigated on agrarose gel.

   D. Gc has been eliminated on starch

   E. The possibility of doing Hp and Gc simultaneously on an acrylamide is being investigated.

   For the Group II systems, AK, ADA, and GPT are still in progress. PGD has been eliminated for statistical reasons, and while GPT is potentially a valuable system and can be detected in fresh blood, the results obtained thus far with GPT on dried blood are unreliable. Of the Group III systems, GSR, Hb, and G6PD have been eliminated.

Four Week Old Stains

The results discussed above for fresh blood are generally applicable to dried stains. The exceptions are that the studies involving acrylamide will not be initiated with dried blood until wet blood optimization results are completed. As with fresh blood, GLO has been eliminated from CAM. GPT gives unreliable results as mentioned above.

Blind Trials

Mr. Wraxall discussed the results obtained from blind trial studies of ESD, EAP, and PGM. The researchs have concluded that the multi-system analysis as discussed in the Statement of Work is the most important and most difficult aspect of the research. They concluded that certain substrates would have to be eliminated so that the multi-system analysis work can be completed efficiently. The following is a summary of the preliminary blind trial conclusions:

ESD It is difficult to distinguish between the results obtained on CAM and starch. However an apparent genetically impossible variant was observed on CAM as a part of the OCJP program. It was re-investigated on starch and found to be a 2-1 phenotype. Repeated analyses on starch and CAM showed the same results. Although the original handling of these samples (form Hawaii) is not known, the results of this fortunate discovery are sufficient to eliminate CAM from being considered for the ESD system.

EAP The inability of EAP to completely separate the a and b bands during electrophoresis with CAM has been noted. This presents a potential problem for mistyping or obtaining inconclusive results. This problem was reflected in the blind trial results. Until a technique is arrived at which can effectively separate these bands on CAM, the use of this substrate should be eliminated as one of the mediums to be considered for EAP.

PGM The PGM system suffers from the same basic problem as EAP. The b and c bands are incompletely separated on CAM. Potential mistyping and inconsistent results may be obtained. The blind trial studies on PGM to date tend to support this conclusion. Thus, until a technique is arrived at using CAM which can unequivocally separate the bands, PGM must be eliminated from consideration on CAM.

Multi-system Analysis

Preliminary studies concerning the simultaneous analysis of several systems on a single substrate have been continuing ahead of schedule:

- Various combinations of PGM, EAP, ESD, and GLO are being investigated on starch and agarose gels.
- Hp and Gc are being investigated on acrylamide
- AK and ADA combinations are feasible on CAM and starch.

Miscellaneous

There were several concerns on the part of the researchers and customer which were expressed:

- There was discussion regarding the interpretation of the Statement of Work. It was agreed that the spirit of the Statement of Work was to get equipment and procedures set up that would be used by crime labs rather than just meeting the letter of the contract.
- It was discovered that two complete nanophore units are not available. The original impression of both Beckman and Aerospace was that two units were fabricated by NASA. The removal of one complete unit left the research project without a functional nanophore. It is expected that the equipment will be returned within one month.

- o There are problems in the visualization of PGM. It is believed that the cause of these problems is due to faulty reagents.
- o There was speculation and questions concerning the best item of equipment to be used for completion of the Statement of Work. These questions arose because of the unavailability of nanophore.
- o Additional results have shown that the clarity of results are a direct relationship to the size of the sample spotted (for CAM) or inserted (for starch).

Action Items

Aerospace will determine the reason for the unavailability of the nanophore units from the laboratory by NASA.

Agreements

- o The only acceptable end product of this research is the development of a system (s) which will benefit the crime laboratories.
- o Blind trials will be repeated using nanophore
- o Equipment selection cannot be made until all research including system optimization and multi-system analysis is completed.

AGENDA

ELECTROPHORESIS DEVELOPMENT
PROGRAM REVIEW
JUNE 20-21, 1977

I. Program Status
   - A. Schedule
   - B. Tasks Completed

II. Isoenzyme Optimization
   - A. Blind Testing
   - B. Optimization and Procedures
   - C. Substrate Screening
   - D. Simultaneous Analysis

III. Equipment Status

IV. Hazardous Materials Report

V. Future Plans

National Institute of Justice
United States Department of Justice
Washington, D.C. 20531

EsD Blind Trial and Detectability
Summary of Results -- Whole Blood

Total Activity	Total Stains x 4	Correct	Incorrect	Inconclusive	No Activity	
250 Ng.	28	9	7	12	--	CAM
125 Ng.	28	13	8	8	4	CAM
60 Ng.	28	18	3	3	4	CAM
30 Ng.	28	3	--	1	24	CAM
250 Ng.	28	24	--	--	4	STARCH
125 Ng.	28	27	--	1	--	STARCH
60 Ng.	28	15	3	10	--	STARCH
30 Ng.	28	10	--	14	4	STARCH

* Results read from Polaroid photograph.

EsD Blind Trial and Detectability
Summary of Results - Dried Stains

Stain	Total Stains x 4	Correct	Incorrect	Inconclusive	No Activity	
2x0.5 cm. 11 days	8	5	--	3	--	CAM
2x0.5 cm. 21 days	8	8	--	--	--	CAM
2x0.5 cm. 28 days	12	9	2	1	--	CAM
1x0.5 cm. 11 days	8	4	1	--	3	CAM
1x0.5 cm. 21 days	8	8	--	--	--	CAM
1x0.5 cm. 28 days	12	6	1	1	4	CAM
2x0.5 cm. 11 days	8	7	1	--	--	STARCH
2x0.5 cm. 21 days	8	5	2	1	--	STARCH
2x0.5 cm. 28 days	12	5	4	2	1	STARCH
1x0.5 cm. 11 days	8	3	1	4	--	STARCH
1x0.5 cm. 21 days	8	6	1	1	--	STARCH
1x0.5 cm. 28 days	12	3	2	7	--	STARCH

EAP Blind Trial and Detectability
Summary of Results -- Whole Blood

Total Activity	Total Stains x 4	Correct	Incorrect	Inconclusive	No Activity	
500 Ng.	28	26	--	2	--	CAM
250 Ng.	56	42	2	12	--	CAM
125 Ng.	28	13	3 †	8	4	CAM
500 Ng.	28	24	--	4	--	STARCH
250 Ng.	49*	32	2 †	12	3	STARCH
125 Ng.	21*	12	--	6	3	STARCH

* Less 1 reader for 7 stains.

† ACD blood sample losing 'A' band activity.

EAP Blind Trial and Detectability

Summary of Results - Dried Stains

Stain	Total Stains x 4	Correct	Incorrect	Inconclusive	No Activity
4x0.5 cm.					
36 days	12	1	3	8	--
25 days	8	5	1	2	--
18 days	8	5	1	2	--
3x0.5 cm.					
36 days	12	2	1	9	--
25 days	8	4	--	--	4
18 days	8	4	--	4	--
2x0.5 cm.					
36 days	12	4	--	8	--
25 days	8	4	1	3	--
18 days	8	6	--	2	--
1x0.5 cm.					
36 days	12	--	--	4	8
25 days	8	2	--	2	4
18 days	8	1	--	7	--

CAM

Stain	Total Stains x 4	Correct	Incorrect	Inconclusive	No Activity
4x0.5 cm.					
36 days	9*	9	--	--	--
25 days	6*	6	--	--	--
18 days	6*	6	--	--	--
3x0.5 cm.					
36 days	12	12	--	--	--
25 days	8	8	--	--	--
18 days	8	8	--	--	--
2x0.5 cm.					
36 days	12	12	--	--	--
25 days	8	8	--	--	--
18 days	8	8	--	--	--
1x0.5 cm.					
36 days	12	11	--	1	--
25 days	8	8	--	--	--
18 days	8	5	--	3	--

STARCH

* Less 1 reader for 7 stains.

GRADIPORE

ACRYLAMIDE

GRADIENT

5-2.5 % HP (1mm 3mm)

HOMOGENEOUS

HP (1mm 3mm)

PGM
EAP ?
E&D
GLO I

BLIND TRIAL
&
DETECTABILITY

ELIMINATION OF SUBSTRATE
(Grad | Homo | Vert. | Horiz)

ELIMINATION OF UNWANTED

C.M.M.

PGM
EAP
E&D
GLO I

Ag-Ab

GC

BLIND TRIAL
&
DETECTABILITY

ELIMINATION OF SUBSTRATE

STARCH

PGM
EAP
E&D
GLO I

→ MULTISYSTEM
or
1 BUFFER SYSTEM
(1 Buffer 1 pH)
1 . 1 2 .
2 . 2 .

BLIND TRIAL
&
DETECTABILITY

D.I. whole Blood
dil. cruor
reduced cloth.

ELIMINATION OF SUBSTRATE

COMBINATION OF
1 or more Syst
w. 1 pH.

( ) = UPDATED FROM PREVIOUS

			CAM				STARCH			ACRYLAMIDE				AGAR			CELLOGEL				AGAROSE				
D.I.	SYSTEM	STAT.	NANOPHOR	MICROZONE	MULTIZONE	SHANDON	NANOPHOR	MULTIZONE	SHANDON	NANOPHOR	MULTIZONE	SHANDON	GRADIPORE	NANOPHOR	MULTIZONE	SHANDON	NANOPHOR	MICROZONE	MULTIZONE	SHANDON	NANOPHOR	MICROZONE	MULTIZONE	SHANDON	
0.47	PGM	IP	✓	✓	✓		IP	✓	✓		(✓)	(✓)	(✓)					(✓)				(IP)	(IP)	(IP)	
0.35	EAP	IP	✓	✓	✓		✓	✓	✓		(✓)	(✓)	(✓)					(✓)				(IP)	(IP)	(IP)	
0.69	ESD	IP	✓	✓	✓			✓	✓		(✓)	(✓)	(✓)									(IP)	(IP)	(IP)	
0.38	GLO	IP	(✓)	(✓)	(✓)	(✓)		✓	✓		(IP)	(IP)	(IP)					(✓)				(IP)	(IP)	(IP)	
0.39	Hp	IP								✓	(✓)	(✓)	✓												✓ BECKMAN ACRYLAMIDE APPR
0.45	Gc	IP	✓	✓	(✓)	(✓)	X	X	X		(✓)	(✓)	(✓)										(✓)	(✓)	
0.82	AK	IP	✓	✓							✓	✓													
0.82	ADA	IP	✓	✓							✓	✓													
0.91	PGD	X	✓	✓							✓	✓													
0.38	GPT	IP		(✓)							(✓)	(✓)						(✓)							
0.95	GSR		(✓)	(✓)	(✓)	(✓)	(✓)																		
0.96	Hb		(✓)	✓	✓																				
0.94	G6PD		(✓)	✓	✓																				
0.93	PCE-E1	X																							
0.92	PCE-E2	X																							
0.96	PEPA	X																							
0.96	CA II	X																							

			CAM				STARCH			ACRYLAMIDE / AGAR							CELLOGEL				AGAROSE				
D.I.	SYSTEM	STAT.	NANOPHOR	MICROZONE	MULTIZONE	SHANDON	NANOPHOR	MULTIZONE	SHANDON	NANOPHOR	MULTIZONE	SHANDON	GRADIPORE	NANOPHOR	MULTIZONE	SHANDON	NANOPHOR	MICROZONE	MULTIZONE	SHANDON	NANOPHOR	MICROZONE	MULTIZONE	SHANDON	
0.47	PGM	IP	✓	✓				✓	✓																
0.35	EAP	IP	✓	✓				✓	✓																
0.61	ESD	IP	✓	✓				✓	✓																
0.38	GLO	IP	⊗	⊗	⊗	⊗		✓	✓																
0.39	Hp	IP					X	X	X	IP	✓	✓	✓												✓ BECKMAN ACRYLAMIDE APN
0.45	Gc	IP	IP	✓			X	X	X		IP	IP	IP										IP	IP	
0.82	AK	IP		✓				✓	✓																
0.82	ADA	IP		IP				✓	✓																
0.91	PGD	X	X	X	X	X	X	X	X																
0.38	GPT	IP	⊗	⊗	⊗	⊗	⊗	⊗	⊗																
0.95	GSR	⊗																							
0.96	IIb	⊗		✓																					
0.94	G6PD	⊗																							
0.93	PCE-E_1	X																							
0.92	PCE-E_2	X																							
0.96	PEPA	X																							
0.96	CA II	X																							

CURRENT STATUS · 20 JUNE 77

PGM	STARCH (OR CAM)	REACTION MIXTURE
. EAP	STARCH	O.K.
. ESD	STARCH (OR CAM)	O.K.
. GLO	STARCH	REACTION - POSITIVE STAINING METHOD
. Hp	ACRYLAMIDE	HORIZONTAL METHOD. REDUCED GRADIENT OR HOMOGENEOUS GEL
.. Gc	CAM (OR ACRYLAMIDE)	REPRODUCIBLE 4-WK OLD DETECTION ON CAM AND ACRYLAMIDE. REDUCED GRADIENT OR HOMOGENEOUS GEL
AK-ADA	STARCH OR CAM	HORIZONTAL ACRYLAMIDE METHOD BLIND TRIALS

II. MULTI-SYSTEM ANALYSIS

A. SUPPORT MEDIA

1. STARCH GEL : VARIOUS COMBINATIONS OF PGM, EAP, ESD, & GLO

2. ACRYLAMIDE GEL : Hp - Gc.

3. STARCH GEL OR CAM : AK - ADA.

4. AGAROSE GEL : VARIOUS COMBINATIONS OF PGM, EAP, ESD, & GLO.

B. DRIED-STAINS

VIII. BLIND TRIALS

A. FINISH PGM

B. GLO

C. Gc

D. Hp

E. AK

F. ADA

THE AEROSPACE CORPORATION

Suite 4040, 955 L'Enfant Plaza, S.W., Washington, D.C. 20024, Telephone: (202) 484-5500

3330-RCS-77-107
July 21, 1977

Mr. Jean Bordeau
Beckman Instruments, Inc.
Advanced Technology Division
1630 South State College Place
Anaheim, California 92806

Dear Jean:

Enclosed are the official minutes of the Bloodstain Electrophoresis Program Review that was held at the White Mountain Research Center, University of California, Berkeley, 20 June 1977.

Please disregard the draft copy which was sent earlier as it included several omissions. These minutes reflect the information that was presented at the meeting.

If I can be of any further assistance, please do not hesitate to call.

Sincerely,

Robert C. Shaler

Robert C. Shaler, Ph. D.
Director
Forensic Sciences

RCS:pl

Enclosure

BLOODSTAIN ANALYSIS SYSTEM
MINUTES OF MEETING WITH BECKMAN/BERKELEY
20 JUNE 1977

The meeting was called to order at 1:15 P.M. (Pacific Standard Time) at the White Mountain Research Center, University of California.

Attendees:

The Aerospace Corporation

G. Denault
S. Derda
R. Kennel
Q. Kwan
G. Roberts
R. Shaler

Beckman Instruments

J. Bordeau
L. Morgan
M. Stolorow
J. Walsh
B. Wraxall

University of California Berkeley

B. Del Ray
G. Harmor

Equipment Optimization

Mr. Bordeau discussed the progress regarding equipment optimization. The nanophore was not used during this period because of its removal from the laboratory by NASA. The reasons for the removal of the equipment was not known, and its return to the laboratory is expected within one month. The other items of equipment: the Multizone and Shandon units are being used extensively; the Gradipore apparatus has extreme limitations in these studies.

System (Isoenzyme) Optimization

Fresh Blood

Mr. Stolorow presented the results to date concerning the optimization the isoenzyme systems.

1. A schematic was presented (Attachment 1) which illustrates the rationale being used in order to eliminate inefficient efforts regarding the use of the different substrates or equipment. The flow diagrams show that various substrates (acrylamide, starch or cellulose acetate membrane (CAM) will be investigated with each system. The system which gives the best results with a particular substrate will then be studied with blind trial results, and detectability limits being used as selection criteria. These studies should then permit a decision to be made regarding the acceptability of one or more substrates for each system. Once the optimization for each system on a particular substrate (s) is determined, the appropriate item of equipment will be selected.
2. An update chart was presented (Attachment 2) which shows the progress regarding the optimization of each system on the different substrates. The chart illustrates that all Group I systems are still being evaluated. The following preliminary conclusions have been arrived at since the previous program review:
   A. PGM, EAP, EsD have been eliminated from acrylamide. This elimination is due to both theoretical considerations and experimental results. The results showed that acrylamide severely inhibits the enzyme activities of these systems.
   B. GLO has been tentatively eliminated on CAM because of poor resolution of the bands.
   C. The above systems are also being investigated on agrarose gel.
   D. Gc has been eliminated on starch
   E. The possibility of doing Hp and Gc simultaneously on an acrylamide is being investigated.

For the Group II systems, AK, ADA, and GPT are still in progress. PGD has been eliminated for statistical reasons, and while GPT is potentially a valuable system and can be detected in fresh blood, the results obtained thus far with GPT on dried blood are unreliable. Of the Group III systems, GSR, Hb, and G6PD have been eliminated.

Four Week Old Stains

The results discussed above for fresh blood are generally applicable to dried stains. The exceptions are that the studies involving acrylamide will not be initiated with dried blood until wet blood optimization results are completed. As with fresh blood, GLO has been eliminated from CAM. GPT gives unreliable results as mentioned above.

Blind Trials

Mr. Wraxall discussed the results obtained from blind trial studies of ESD, EAP, and PGM. The researchers have concluded that the multi-system analysis as discussed in the Statement of Work is the most important and most difficult aspect of the research. They decided that certain substrates would have to be eliminated so that the multi-system analysis work can be completed efficiently. The following is a summary of the preliminary blind trial conclusions:

ESD — It is difficult to distinguish between the results obtained on CAM and starch. However an apparent genetically impossible variant was observed on CAM as a part of the OCJP program. It was re-investigated on starch and found to be a 2-1 phenotype. Repeated analyses on starch and CAM showed the same results. The original handling of these samples (from Hawaii) is not known.

EAP — The inability of EAP to completely separate the a' and b bands during electrophoresis with CAM has been noted. This presents a potential problem for mistyping or obtaining inconclusive results. This problem was reflected in the preliminary blind trial results.

PGM — The PGM system suffers from the same basic problem as EAP. The b and c bands are incompletely separated on CAM. Potential mistyping and inconsistent results

may be obtained. The incompleted blind trial studies on PGM to date tend to support this conclusion.

Multi-system Analysis

Preliminary studies concerning the simultaneous analysis of several systems on a single substrate have been continuing ahead of schedule:

- o Various combinations of PGM, EAP, ESD, and GLO are being investigated on starch and agarose gels.
- o Hp and Gc are being investigated on acrylamide AK and ADA combinations are feasible on CAM and starch.

Hazardous Materials

The list of hazardous materials sent to Mr. Bordeau by Mr. Roberts was reviewed. The discussion was centered around warning the eventual users of this research about the potential hazards of the materials. The Berkeley laboratory is aware of these materials and will be careful with their use.

Miscellaneous

There were several concerns on the part of the researchers and customer which were expressed:

- o There was discussion regarding the interpretation of the Statement of Work. It was agreed that the spirit of the Statement of Work was to get equipment and procedures set up that would be used by crime labs rather than just meeting the letter of the contract.
- o It was discovered that two complete nanophore units are not available. The original impression of both Beckman and Aerospace was that two units were fabricated by NASA. The removal of one complete unit left the research project without a functional nanophore. It is expected that the equipment will be returned within one month.

- There are problems in the visualization of PGM. It is believed that the cause of these problems is due to faulty reagents.
- There was speculation and questions concerning the best item of equipment to be used for completion of the Statement of Work. These questions arose because of the unavailability of nanophore.
- Additional results have shown that the clarity of results are a direct relationship to the size of the sample spotted (for CAM) or inserted (for starch).

Action Items

Aerospace will determine the reason for the unavailability of the nanophore units from the laboratory by NASA.

Agreements

- The only acceptable end product of this research is the development of a system (s) which will benefit the crime laboratories.
- Blind trials will be repeated using nanophore
- Equipment selection cannot be made until all research including system optimization and multi-system analysis is completed.

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EsD Blind Trial and Detectability
Summary of Results -- Whole Blood

tal ivity	Total Stains x 4	Correct	Incorrect	Inconclusive	No Activity	
) Ng.	28	9	7 [ ]	12	--	CAM
5 Ng.	28	13		8	4	
Ng.	28	18	3 [ ]	3	4	
Ng.	28	3	--	1	24	
) Ng.	28	24	--	--	4	STARCH
5 Ng.	28	27	--	1	--	
Ng.	28	15	3]*	10	--	
Ng.	28	10	--	14	4	

* Results read from Polaroid photograph.

EsD Blind Trial and Detectability
Summary of Results - Dried Stains

h	Total Stains x 4	Correct	Incorrect	Inconclusive	No Activity	
0.5 cm. days	8	5	--	3	--	CAM
0.5 cm. days	8	8	--	--	--	
0.5 cm. days	12	9	2 [ ]	1	--	
0.5 cm. days	8	4	1 [ ]	--	3	
0.5 cm. days	8	8	--	--	--	
0.5 cm. days	12	6	1 [ ]	1	4	
0.5 cm. days	8	7	1 [ ]	--	--	STARCH
0.5 cm. days	8	5	2 [ ]	1	--	
0.5 cm. days	12	5	4 [ ]	2	1	
0.5 cm. days	8	3	1 [ ]	4	--	
0.5 cm. days	8	6	1 [ ]	1	--	
0.5 cm.	12	3	2 [ ]	7	--	

EAP Blind Trial and Detectability
Summary of Results -- Whole Blood

l vity	Total Stains x 4	Correct	Incorrect	Inconclusive	No Activity	
Ng.	28	26	--	2	--	CAM
Ng.	56	42	2	12	--	
g.	28	13	3 [ ]†	8	4	
3.	28	24	--	4	--	STARCH
1.	49*	32	2 †	12	3	
..	21*	12	--	6	3	

1 reader for 7 stains.

lood sample losing 'A' band activity.

EAP Blind Trial and Detectability

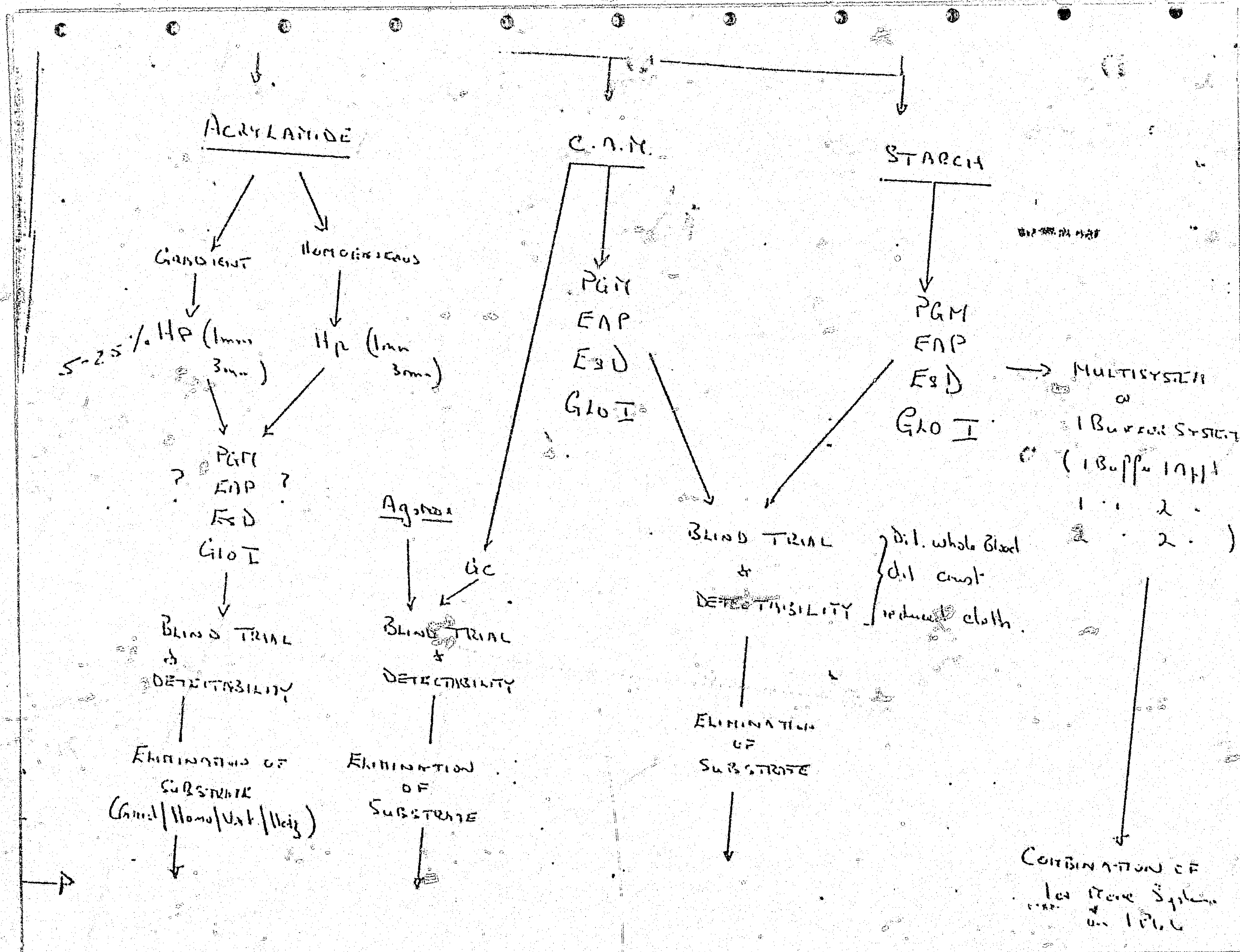
Summary of Results - Dried Stains

n	Total Stains x 4	Correct	Incorrect	Inconclusive	No Activity
5 cm.					
ays	12	1	3		
ays	8	5	1	8	--
ays	8	5	1	2	--
				2	--
5 cm.					
ays	12				
ays	8	2	1		
ays	8	4	--	9	--
		4	--	--	4
				4	--
5 cm.					
ays	12	4			
ays	8	4	--	8	
ays	8	5	1	3	--
			--	2	--
cm.					
ays	12				
	8	2	--	4	8
	8	1	--	2	4
				7	--
cm.					
ys	Ks*	9			
ys	6*	6	--	--	--
ys	6*	6	--	--	--
			--	--	--
cm.					
ys	12	12			
ys	8	8	--	--	--
ys	8	8	--	--	--
			--	--	--
cm.					
ys	12	12			
ys	8	8	--	--	--
ys	8	8	--	--	--
			--	--	--
cm.					
s	12	11			
s	8	8	--	1	--
s	8	5	--	--	--
			--	3	--

C A M

S T A R C H

1 reader for 7 stains.



ACRYLAMIDE
GRADIENT
HOMOGENEOUS
5-25% HP (1mm 3mm)
Hp (1mm 3mm)
PGM
EAP
EsD
Glo I
BLIND TRIAL & DETECTABILITY
ELIMINATION OF SUBSTRATE
Agarose
BLIND TRIAL & DETECTABILITY
ELIMINATION OF SUBSTRATE
C.A.M.
PGM
EAP
EsD
Glo I
STARCH
PGM
EAP
EsD
Glo I
MULTISYSTEM
or
1 Buffer System
BLIND TRIAL & DETECTABILITY
dil. whole blood
dil crust
reduced cloth
ELIMINATION OF SUBSTRATE
COMBINATION OF

T.	SYSTEM		STAT	CATH: HANDPHOR	CATH: MICROZONE	CATH: MULTIZONE	CATH: SHANDON	STARCH: HANDPHOR	STARCH: MULTIZONE	STARCH: SHANDON	ACRYLAMIDE: HANDPHOR	ACRYLAMIDE: MULTIZONE	ACRYLAMIDE: SHANDON	ACRYLAMIDE: GRADIZONE	AGAR: HANDPHOR	AGAR: MULTIZONE	AGAR: SHANDON	CELLULOSE: HANDPHOR	CELLULOSE: MICROZONE	CELLULOSE: MULTIZONE	CELLULOSE: SHANDON	AGAROSE: HANDPHOR	AGAROSE: MICROZONE	AGAROSE: MULTIZONE	AGAROSE: SHANDON	
.17	IGM		IP	✓	✓	✓		IP	✓	✓		(IP)	(IP)	(IP)					(✓)				(IP)	(IP)	(IP)	
.35	EAP		IP	✓	✓	✓		✓	✓	✓		(IP)	(IP)	(IP)					(✓)				(IP)	(IP)	(IP)	
.09	ESD		IP	✓	✓	✓			✓	✓		(IP)	(IP)	(IP)									(IP)	(IP)	(IP)	
.26	GLO		IP	(IP)	(IP)	(IP)	(IP)		✓	✓		(IP)	(IP)	(IP)					(IP)				(IP)	(IP)	(IP)	
.39	Hp		IP								✓	(✓)	(✓)	✓												✓ BECKMAN ACRYLAMIDE APPARATUS
.45	Gc		IP	✓	✓	(✓)	(✓)	X	X	X		(✓)	(✓)	(✓)										✓	✓	
.82	AK		IP	✓	✓				✓	✓																
.62	ADA		IP	✓	✓				✓	✓																
.41	PGD		X	✓	✓				✓	✓																
.38	GPT		IP		(✓)				(✓)	(✓)									(✓)							
.15	GSR		(X)	(X)	(X)	(X)	(X)																			
.96	IIIb		(X)	✓	✓																					
.94	GGPD		(X)	✓	✓																					
.13	PCE-E1		X																							
.32	PCE-E2		X																							
.6a	PEPA		X																							
.6	CA IC		X																							

			CAM				SHANGH			ACETAMID				ABAK			CELLUCEL				ABMOUSE				
T.	SYSTEM	STAT.	MANOPHOR	MICROZONE	MULTIZONE	SHANDON	MANOPHOR	MULTIZONE	SHANDON	MANOPHOR	MULTIZONE	SHANDON	GRADIPORE	MANOPHOR	MULTIZONE	SHANDON	MANOPHOR	MICROZONE	MULTIZONE	SHANDON	MANOPHOR	MICROZONE	MULTIZONE	SHANDON	
17	PGM	IP.	✓	✓				✓	✓																
35	EAP	IP	✓	✓				✓	✓																
69	ESD	IP	✓	✓				✓	✓																
38	GLO	IP	⊗	⊗	⊗	⊗		✓	✓																
31	IIp	IP					X	X	X	IP	⊘	⊘	✓												✓ BECKMAN ACRYLAMIDE MIXTURE
15	Gc	IP	IP	✓			X	X	X		⊗	⊗	⊗										IP	IP	
82	AK	IP		✓				✓	✓																
42	ADA	IP		IP				✓	✓																
91	PGD	X	X	X	X	X	X	X	X																
28	GPT	IP	⊗	⊗	⊗	⊗	⊗	⊗	⊗																
15	GSR	⊗																							
36	IIb	⊗		✓																					
41	G6PD	⊗																							
3	PCE-E1	X																							
2	PCE-E2	X																							
	PEPA	X																							
	CA II	X																							

CURRENT STATUS · 20 JUNE 77

PGM	STARCH (OR CAM)	REACTION MIXTURE
EAP	STARCH	O.K.
ESD	STARCH (OR CAM)	O.K.
GLO	STARCH	REACTION - POSITIVE STAINING METHOD
Hp	ACRYLAMIDE	HORIZONTAL METHOD REDUCED GRADIENT OR HOMOGENEOUS GEL
Gc	CAM (OR ACRYLAMIDE)	REPRODUCIBLE 4-WK OLD DETECTION ON CAM AND ACRYLAMIDE REDUCED GRADIENT OR HOMOGENEOUS GEL
AK-ADA	STARCH OR CAM	HORIZONTAL ACRYLAMIDE METHOD BLIND TRIALS

II. MULTI-SYSTEM ANALYSIS

A. SUPPORT MEDIA

1. STARCH GEL : VARIOUS COMBINATIONS OF PGM, EAP, ESD, & GLO
2. ACRYLAMIDE GEL : Hp - Gc.
3. STARCH GEL OR CAM : AK - ADA.
4. AGARDSE GEL : VARIOUS COMBINATIONS OF PGM, EAP, ESD, & GLO.

B. DRIED-STAINS

VIII. BLIND TRIALS

A. FINISH PGM
B. GLO
C. Gc
D. Hp
E. AK
F. ADA

THE AEROSPACE CORPORATION

Suite 4040, 955 L'Enfant Plaza, S.W., Washington, D.C. 20024, Telephone: (202) 484-5500

3330-RCS-77-111
August 2, 1977

Mr. Jean Bordeau
Beckman Instruments, Inc.
Advanced Technology Division
1630 South State College Place
Anaheim, California 92806

Dear Jean:

Enclosed please find the agenda for the Tuesday, August 9, 1977 Program Review Meeting.

We expect to arrive in San Francisco around 11:30 A.M. and it would be convenient to have the meeting start at 1:00 P.M.

Sincerely,

Robert C. Shaler
Director
Forensic Sciences

RCS:pl

Enclosure

cc: L. Morgan
M. Stolorow
J. Walsh
B. Wraxall
B. Grunbaum
B. Del Ray
G. Harmor

BECKMAN-BERKELEY BLOOD ELECTROPHORESIS PROGRAM REVIEW

AGENDA

AUGUST 9, 1977 1:00 P. M.

This program review represents perhaps the most important thus far and concrete decisions must be made regarding the selection of substrates for the genetic systems being considered. These selections must be correct since the successful and timely completion of this research and the future utility of the systems which are developed have to benefit the crime laboratories. In order to make these decisions all the relevant data must be put into perspective and reviewed simultaneously. Thus all conclusions made regarding the future direction of this program must be substantiated by an in-depth presentation and discussion of the data.

System Optimization (Selection of Candidates and Substrates)

The substrates, CAM, starch, acrylamide and agarose will be discussed for each genetic system in all three priority groups. All relevant test data or non-test logic must be presented which demonstrates the suitability of each particular substrate for each genetic system. The data should demonstrate conclusively the reasoning behind the selections to be made.

The results of both blind trial studies will be presented and discussed in order to assess their meaning, validity and utility in selecting specific substrates.

All original data should be available in case questions should arise.

Multi-system Analysis Concept (System Development)

It is expected that the selection of substrates will have been made with multi-system analysis phase of the research firmly in mind. Thus any data which has been obtained regarding multi-system concept development will be discussed from the viewpoint of the chosen systems and substrates. The discussion will concentrate on the choice of systems for simultaneous analysis, the rationale of the approach being used, the technical feasibility of the concept and the practicality of the developed concept in crime laboratories.

THE AEROSPACE CORPORATION

Suite 4040, 955 L'Enfant Plaza, S.W., Washington, D.C. 20024, Telephone: (202) 484-5500

3330-RCS-77-025
August 17, 1977

Mr. Jean Bordeau
Beckman Instruments, Inc.
Advanced Technology Division
1630 South State College Place
Anaheim, California 92806

Dear Jean:

Enclosed are the official minutes of the Bloodstain Electrophoresis Program Review that was held the White Mountain Research Center, University of California, Berkeley, 9 August 1977.

These minutes reflect the information that was presented at the meeting.

If I can be of any further assistance, please do not hesitate to call.

Sincerely,

Robert C. Shaler

Robert C. Shaler, Ph. D.
Director
Forensic Sciences

RCS:pl

Enclosures

PROGRAM REVIEW MEETING
ELECTROPHORETIC BLOODSTAIN ANALYSIS
BECKMAN/UNIVERSITY OF CALIFORNIA
9 AUGUST 1977

ATTENDEES

Aerospace	Beckman	University of California
G. Denault	J. Bordeau	B. Grunbaum
S. Derda	L. Morgan	
Q. Kwan	J. Walsh	
R. Shaler	B. Wraxall	

PROGRAM OVERVIEW AND STATUS

The first portion of the meeting was devoted to a formal presentation by J. Bordeaux on the current status of the program, (see attachments). While much of the material had been presented over the course of previous meetings, the presentation served as a concise history of the effort and accomplishments to date and detailed future plans for the upcoming system development phase. Salient points of the presentation are summarized below:

SYSTEM DEFINITION

The system definition phase of the program is on schedule and will be concluded by 1 September as originally planned. The blood constituents selected for development yield the required 1:200 discrimination index. By continuing efforts conducted directly on this program and previous efforts of the researchers, the substrates accepted to date are starch gel, cellulose acetate membrane and acrylamide gel. Methodologies to run the various constituents on these substrates have been defined. The testing protocol and results of blind trial tests run on EAP, ESD, and PGM were presented.

A summary of the System Definition work performed thus far is as follows:

Equivalent	Starch Gel	CAM	Acrylamide
ESD	GLOI	Gc	Hp
PGM	EAP		
AK	ADA		

Current conclusions indicate that a 1:200 discrimination probability is achievable as is the requirement for use of no more than three (3) setups. Interpretation of results by experienced readers is unequivocal, however aids may be required to assist crime laboratory technicians.

SENSITIVITY ANALYSIS

A very brief presentation was made regarding sensitivity analysis (i.e., that sample level at which unequivocal interpretations can no longer be achieved), however, it was decided to delay any further discussion of this subject until a future date.

SYSTEM DEVELOPMENT

Work on the system development portion of the program will begin as scheduled on 1 September. This effort will concentrate on combining compatible blood constituents to minimize operator effort, time, and cost.

After the formal presentation was concluded, each of the selected blood constituents was discussed individually with Aerospace personnel reviewing actual electrophoretic plates and photographs with the researchers.

Some preliminary work has been done on multiple system optimization and results appear to be encouraging. Multiple system optimization will be pursued in depth during the system development phase of the program.

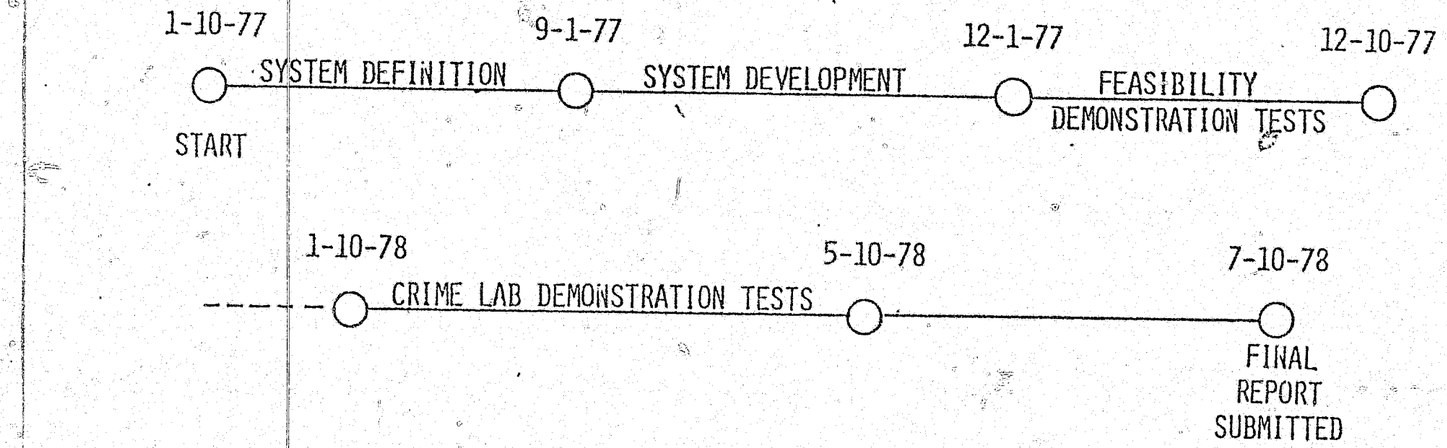
PROGRAM REVIEW

BLOODSTAIN ANALYSIS

AUGUST 9, 1977

BECKMAN

PROGRAM MAJOR MILESTONES



SYSTEM DEFINITION

- SELECT CONSTITUENTS
- DEVELOP METHODOLOGIES
- SELECT SUBSTRATES

CONSTITUENTS CONSIDERED

FROM STATEMENT OF WORK

HEMOGLOBIN, Hb
HAPTOGLOBIN, Hp
GROUP SPECIFIC COMPONENTS
GLUTAMATE PYRUVATE TRANSAMINASE, GPT
RED CELL ACID PHOSPHATASE, EAP
PHOSPHOGLUCOMUTASE, PGM
ADENYLATE KINASE, AK
ADENOSINE DEAMINASE, ADA
PSEUDOCHOLINESTERASE (E2), PCE (E2)
6 PHOSPHOGLUCONATE DEHYDROGENASE, 6 PGD
GLUCOSE 6 PHOSPHATE DEHYDROGENASE, G6PD
ESTERASE D, EsD
PEPTIDASE A, PEP-A
CARBONIC ANHYDRASE

ADDITIONAL CONSTITUENTS CONSIDERED

AMYLASE
URIDINE PHOSPHOKINASE
GLYOXYLASE I
GSR
BIS-ALBUMIN
TRANSFERRIN
CERRULOPLASMIN
ALPHA-1 ANTITRYPSIN

RATIONALE FOR SELECTION OF CONSTITUENTS

- DISCRIMINATION INDEX
- KNOW STABILITY IN STAINS
- AVAILABLE METHODOLOGY
- UNAMBIGUITY OF PHENOTYPES

CONSTITUENTS SELECTED FOR DEVELOPMENT

BLOOD CONSTITUENTS	DI
HAPTOGLOBIN, Hp	0.39
GROUP SPECIFIC COMPONENTS	0.45
RED CELL ACID PHOSPHATASE, EAP	0.35
PHOSPHOGLUCOMUTASE, PGM	0.47
ADENYLATE KINASE, AK	0.82
ADENOSINE DEAMINASE, ADA	0.82
ESTERASE D, EsD	0.69
GLYOXYLASE I	0.38

METHODOLOGIES

SEPARATION TECHNIQUE

ELECTROPHORESIS – CONSTANT pH
ISOELECTRIC FOCUSING – GRADIENT pH
CROSSED ELECTROPHORESIS

IDENTIFICATION

SPECIFIC AFFINITY STAINING – FORMAZAN
– STARCH IODIDE
IMMUNOLOGICAL REACTION
IMMUNOFIXATION – ANTIGEN/ANTIBODY

CELLULOSE ACETATE METHODOLOGIES
(INDIVIDUAL ANALYSIS)

	VOLTS	mA	TIME (MIN)	POS	(μℓ) VOLUME	MEMBRANE	BUFFERS TANK	PH
EAP	250	1.5-3.0	50	3	.50	1:5	0.015M TRISODIUM CITRATE 0.0134M SODIUM PHOSPHATE	5.9
PGM	200	0.2-2.0	60	4	.25	1:20	0.1M TRIS 0.1M MALEIC ACID 0.01M MAGNESIUM CHLORIDE 0.01M EDTA (DISODIUM SALT)	7.4
EsD	200	1.5-4.0	60	1	.50	1:7	0.1M TRIS 0.1M MALEIC ACID 0.01M MAGNESIUM CHLORIDE 0.01M EDTA (DISODIUM SALT)	7.4
AK	300	1.5-3.0	45	7	.25	NO DILUTION	0.014M PHOSPHATE	6.25
ADA	250	0.5-2.0	25	3	.25	1:15	0.01M PHOSPHATE	6.5
Gc	500	1.5-3.0	20	1	.25	NO DILUTION	0.0372M TRIS 0.29M GLYCINE	8.4

STARCH GEL METHODOLOGIES

	VOLT	TIME HR	GEL	BUFFERS TANK	pH
EAP	400	4	1:100	0.24M SODIUM PHOSPHATE 0.15M TRISODIUM CITRATE	5.9
PGM	115	16	1:15	TRIS MALEIC ACID EDTA $MgCL_2$	7.4
EsD	300	2	TRIS CITRIC ACID BORIC ACID LiOH	BORIC ACID LiOH	7.2 – TANK 7.4 – GEL
AK	250	4	SUCCINIC ACID TRIS	CITRIC ACID NaOH	4.9 – TANK 5.0 – GEL
ADA	60	16	1:10	0.1M PHOSPHATE $Na\ H_2\ PO_4$ $Na\ H\ PO_4$	6.5
GLO-I	300	4	1:10	0.2M PHOSPHATE	6.8

ACRYLAMIDE GEL METHODOLOGY

	VOLTS	EST MA	TIME (MIN)	POS	(μℓ) VOLUME	BUFFERS GEL	TANK	pH
IIP	100 V	5-1.2	16 HRS	(-)	1-5	0.076M TRIS 0.005M CITRIC ACID	BORIC ACID	GEL 8.6 TANK 6.6

SPECIAL METHODOLOGIC CONSIDERATIONS

- CHLOROFORM
- CLELAND'S REAGENT
- GLYCEROL
- EAP
- EsD

CROSSED ELECTROPHORESIS – Gc/Hp

SUBSTRATE SELECTION

SUBSTRATES CONSIDERED

- STARCH GEL
- CELLULOSE ACETATE MEMBRANE
- ACRYLAMIDE GEL
- AGAROSE GEL
- CELLO GEL
- AGAR
- SILICA GEL

SELECTION LIMITATION

- MOLECULAR SIZE SEPARATION
- CHARGE SEPARATION
  - ELECTROPHORETIC MOBILITY

SUBSTRATES ACCEPTED

- STARCH GEL
- CELLULOSE ACETATE MEMBRANE
- ACRYLAMIDE GEL

BLIND TRIAL TESTS

PURPOSE

TO DETERMINE RELATIVE ACCURACY OF PHENOTYPING UNDER STANDARDIZED CONDITIONS

PROTOCOL

- LOT SIZE – AT LEAST 10 STAINS
- STAIN AGE – 4 WEEKS MINIMUM
- READER – 4 OR MORE WHEN POSSIBLE
- SAMPLE USED – 25$\mu\ell$ STAIN ON CLOTH AGED 4 WEEKS (DIVIDED EQUALLY)

  EQUIPMENT USED:

  - SHANDON-STARCH
  - MICROZONE-CAM

BLIND TRIALS

SYSTEMS TESTED: EAP: ESD: PGM

NUMBER OF TRIALS EACH SYSTEM:

	STARCH GEL	CAM
EAP	2	2
ESD	2	3
PGM	1	1

RESULTS OF BLIND TRIALS - FINAL READINGS
SYSTEM: EAP

		STARCH GEL	CAM
1.	AGE OF STAINS	4 WKS	4 WKS
2.	NUMBER OF STAINS	15	31
3.	NUMBER OF READERS	4	4
4.	TOTAL NUMBER OF READINGS	64	109
5.	NUMBER CORRECT	64	106
6.	NUMBER INCORRECT*	0	3 all 2., called BA, B
7.	NUMBER QUESTIONED	0	3
8.	VARIANTS PRESENT	2 (B & BA)	2

*ONE READER

RESULTS OF BLIND TRIALS - FINAL READINGS
SYSTEM: ESD

		STARCH GEL	CAM
1.	AGE OF STAINS	4 WKS	4 WKS
2.	NUMBER OF STAINS	16	15
3.	NUMBER OF READERS	3	5
4.	TOTAL NUMBER OF READINGS	48	75
5.	NUMBER CORRECT	48	75
6.	NUMBER INCORRECT	0	0
7.	NUMBER QUESTIONED	0	2
8.	VARIANTS PRESENT	2 1-1, 2-1	2

RESULTS OF BLIND TRIALS - FINAL READINGS SYSTEM:PGM

		STARCH GEL	CAM
1.	AGE OF STAINS	4 WKS	4 WKS
2.	NUMBER OF STAINS	16	15
3.	NUMBER OF READERS	4	6
4.	TOTAL NUMBER OF READINGS	64	80
5.	NUMBER CORRECT	64	79
6.	NUMBER INCORRECT	0	1 (what was the phenotype?)
7.	NUMBER QUESTIONED	1	5
8.	VARIANTS PRESENT	3	2 1-1, 2-1

SENSITIVITY ANALYSIS

OBJECTIVE:

TO DETERMINE SAMPLE LEVEL AT WHICH UNEQUIVOCAL INTERPRETATIONS CANNOT BE ACHIEVED

RATIONALE FOR REQUIREMENT:

25 MICROLITERS OF BLOOD MAY BE DIFFICULT TO LOCATE (ESPECIALLY ON CLOTHING OR PERSON OF SUSPECT--E.G., FROM FINE SPRAY OF BLOOD)

SENSITIVITY ANALYSIS (CONTD)

ANALYTICAL CONSIDERATIONS:

- EFFECT OF GEOMETRY ON RESOLUTION
- DILUTION EFFECTS WITH EXTRACTION
- CAPABILITY FOR RELATIVE QUANTITATION

SENSITIVITY ANALYSIS (CONTD)

METHODOLOGIC CONSIDERATIONS:

- ANALYSIS MUST BE PERFORMED ON ALL SYSTEMS USING 4-WEEK STAINS @ SEVERAL (E.G., 4) DECREASING LEVELS
- SUFFICIENT ANALYSES MUST BE PERFORMED TO ALLOW INTERPRETATION OF RESULTS
- SAMPLES SHOULD REPRESENT ALL PHENOTYPES TO REVEAL DIFFERENTIAL SENSITIVITY PROBLEMS

SENSITIVITY ANALYSIS (CONTD)

PROGRAMMATIC CONSIDERATIONS:

- INVESTIGATION OF SENSITIVITY PARAMETER REPRESENTS DIVERSION FROM CRITICAL PATH
- INVESTIGATION WOULD REQUIRE SCHEDULE RELIEF
- INVESTIGATION WOULD HAVE TBD COST IMPACT

SENSITIVITY ANALYSIS (CONTD)

RECOMMENDATIONS:

DELAY INVESTIGATIONS UNTIL COMBINATION TRIALS ARE COMPLETE

- OTHER FACTORS MAY BE FORCING FUNCTION
- NUMBER OF REQUIRED ANALYSES MAY BE REDUCED
- MINIMIZING COST AND SCHEDULE IMPACT

SUMMARY OF SYSTEM DEFINITION

EQUIVALENT	STARCH GEL	CAM	ACRYLAMIDE
EsD	GLO 1	Gc	IIP
PGM	EAP		
AK	ADA		

YIELDS UNAMBIGUOUS RESULTS

CAN BE DONE WITH LESS THAN 5 MANHOURS OF EFFORT IN 18 HOURS

ACHIEVE 1 IN 200 PROBABILITY OF DISCRIMINATION

CONCLUSIONS - SYSTEM DEFINITION

- 1:200 DISCRIMINATION POTENTIAL IS ACHIEVABLE
- INTERPRETATION OF RESULTS BY EXPERIENCED READERS IS UNEQUIVOCAL; AIDS MAY BE REQUIRED TO ASSIST CRIME LAB WORKERS - photographs etc.
- REQUIREMENT FOR USE OF NO MORE THAN 3 SETUPS APPEARS ACHIEVABLE
- SYSTEM DEVELOPMENT EFFORT CAN BEGIN WITH HIGH DEGREE OF CONFIDENCE

PLAN FOR SYSTEM DEVELOPMENT

PURPOSE: COMBINE SYSTEM TO MINIMIZE OPERATOR EFFORT, TIME, AND EXPENSE

REQUIRES: COMPROMISE OF IDEAL CONDITIONS FOR ANY SINGLE SYSTEM

PROBLEM: DETERMINE WHETHER ANALYSIS AT LESS THAN OPTIMUM CONDITIONS YIELDS ACCURATE PHENOTYPING

PLAN FOR SYSTEM DEVELOPMENT (CONTD)

METHOD: SELECT MOST PROBABLE COMPATIBLE SYSTEMS

- pH
- BUFFER COMPOSITION
- SEPARATION TIME
- SEPARATION DISTANCE
- VOLTAGE

DETERMINE ACCEPTABLE RANGE OF SYSTEM VARIABLES

EMPIRICAL INVESTIGATION WITHIN THIS RANGE TO DETERMINE ABILITY TO ACHIEVE UNAMBIGUOUS INTERPRETATION

PROTOCOL: PRELIMINARY STUDIES – WHOLE BLOOD

CONFIRMATION STUDIES – BLOOD STAINS

INTEGRATION OF CHEMISTRY AND HARDWARE

- HARDWARE SELECTION
- HARDWARE DEVELOPMENT

THE AEROSPACE CORPORATION

Suite 4040, 955 L'Enfant Plaza, S.W., Washington, D.C. 20024, Telephone: (202) 484-5500

3330-RCS-77-030
17 October 1977

Mr. Jean Bordeau
Beckman Instruments, Inc.
Advanced Technology Division
1630 South State College Place
Anaheim, California 92806

Dear Jean:

Enclosed are the official minutes of the Bloodstain Electrophoresis Program Review that was held at the White Mountain Research Center, University of California, Berkeley, October 3, 1977.

These minutes reflect the information that was presented at the meeting. If I can be of any further assistance, please do not hesitate to call.

Sincerely,

Robert C. Shaler

Robert C. Shaler, Ph. D.
Director
Forensic Sciences

RCS:pl

Enclosure

PROGRAM REVIEW MEETING
ELECTROPHORETIC BLOODSTAIN ANALYSIS
BECKMAN/UNIVERSITY OF CALIFORNIA
3 OCTOBER 1977

Attendees

Aerospace	Beckman	University of California
G. Denault	J. Bordeau	B. Grunbaum
S. Derda	L. Morgan	
Q. Kwan	J. Walsh	
R. Shaler	B. Wraxall	

PROGRAM REVIEW AND STATUS

The System Development portion of the overall program began as scheduled on 1 September 1977. Based on the work performed during the System Definition phase and the Statement of Work requirements calling for no more than three (3) electrophoretic set-ups, the major thrust of the System Development effort will be the intensive further development of the starch/agarose substrate. This substrate was selected as it shows applicability to the largest number of selected constituents and also shows good potential for multisystem analysis.

SYSTEM DEVELOPMENT

1. Confirmation Tests

   A series of tests have been conducted which confirm the results obtained in the Blind Trails conducted during System Definition. These tests were performed using systems and methodologies developed during System Definition.

2. Simultaneous Analysis (Serial Separation)

Selected constituents have been grouped for further development and preliminary results are as follows:

- o Group I - PGM, EsD, GLO1: high degree of confidence for use as a practical system
- o Group II - EAP, AK, ADA: EAP and AK are acceptable now with a good probability of including ADA
- o Group III - Hp, Gc: considered possible, however considerable additional work still required

3. Sequential Analysis (Parallel Separation)

While results obtained to date include good probability that program requirements can be met using the simultaneous analysis system described above, effort is also being directed toward the development of a system utilizing sequential analysis. This effort will be continued until such time as the simultaneous analysis system has been fully developed and the potential for system compromises has been eliminated.

Details of the confirmation tests, simultaneous and sequential analyses are contained in the attached handout which was presented at the meeting.

4. Equipment Selection

Based upon results obtained to date, possible choices of equipment have been identified and availability and pricing data on the potential equipment has been collected. Final equipment selection, however, is system dependent.

FEASIBILITY DEMONSTRATION TEST PLAN

Beckman will submit to Aerospace a draft Feasibility Demonstration Test Plan containing their proposed plan for the content and conduct of the test. Prior to submission of this draft plan, however, the following agreements were reached.

- o To establish Beckman/Berkeley confidence in the referee methods and laboratory chosen by Aerospace for the Feasibility Demonstration Test, fresh blood samples will be analyzed by both Beckman and the referee lab prior to actual test. Results of this fresh blood analysis will be compared to determine if discrepancies exist and the reasons for any discrepancies.
- o Aerospace will obtain the cloth for the samples to be analyzed at the Feasibility Demonstration Test from the Berkeley Laboratory.
- o Samples to be analyzed for the Feasibility Demonstration Test will contain no anti-coagulant.

CRIME LABORATORY DEMONSTRATION TEST PLAN:

Beckman will submit to Aerospace a draft Crime Laboratory Demonstration Test Plan containing their proposed plan for the conduct of the Crime Laboratory testing portion of the program including training methods to be employed for the four labs chosen to participate in the test.

Draft copies of both the Feasibility Demonstration Test Plan and the Crime Laboratory Demonstration Test Plan will be submitted to Aerospace by 24 October 1977.

ACTION ITEMS AND AGREEMENTS

As stated above.

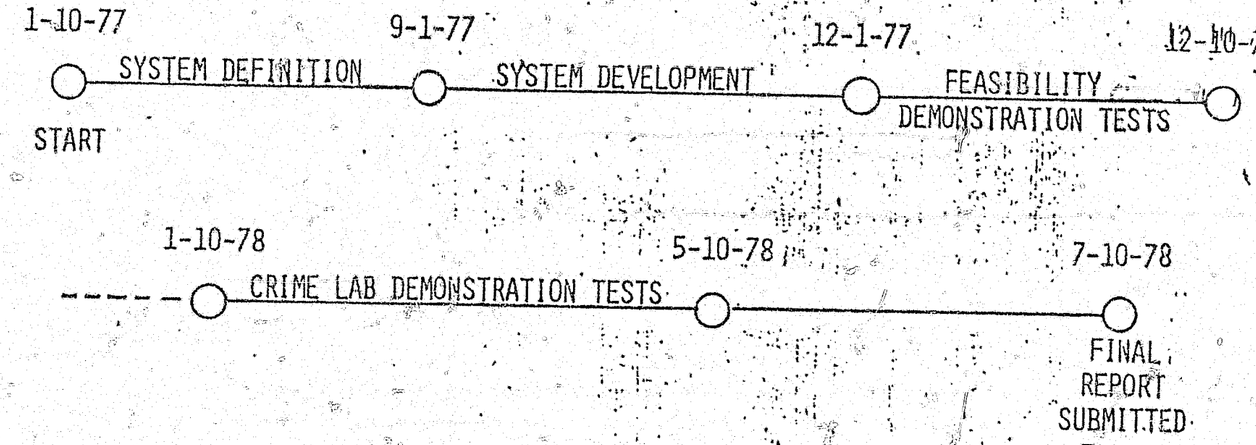
PROGRAM REVIEW

BLOODSTAIN ANALYSIS

OCTOBER 3, 1977

BECKMAN

PROGRAM MAJOR MILESTONES



BECKMAN

MAJOR TASKS - SYSTEM DEVELOPMENT

1. RUN CONFIRMATION TESTS
2. REVIEW SUBSTRATE SELECTION
3. REVIEW SYSTEM VARIABLES

   ESTABLISH GROUPS OF CONSTITUENTS ON:

   - STARCH
   - CAM
   - ACRYLAMIDE
   - AGAROSE

4. ESTABLISH LIMITS FOR PROCESS VARIABLES

   - pH
   - BUFFER COMPOSITION
   - SEPARATION TIME
   - SEPARATION DISTANCE
   - VOLTAGE/AMPERES

5. REVIEW DATA AND SELECT COMPROMISE VALUES FOR THE PROCESS VARIABLES FOR EACH GROUP OF CONSTITUENTS
6. RUN A SERIES OF TESTS WITH EACH GROUP TO DETERMINE ABILITY TO ACHIEVE ACCURATE PHENOTYPING

   - WHOLE BLOOD
   - STAINS

National Institute of Justice
United States Department of Justice
Washington, D.C. 20531

CONFIRMATION TESTS

CONSTITUENT	NUMBER OF STAINS ANALYZED	NUMBER OF VARIANTS	NUMBER OF READERS/ READINGS	NUMBER QUESTIONED CAM	NUMBER QUESTIONED STARCH	NUMBER CORRECT CAM	NUMBER CORRECT STARCH	NUMBER INCORRECT CAM	NUMBER INCORRECT STARCH
EsD	7	3	4-28 CAM 4-27 STARCH	1	0	28	24	0	3
PGM	7	3	4-28	1	0	28	28	0	0
EAP	7	5	4-28	2	0	28	28	0	0
AK	7	1	4-28	0	0	28	28	0	0
ADA	7	2	4-28	1	0	27	28	1	0
GLO-I	7	2	4-25	-	3	-	24	-	1
Gc	7	3	1-4 4-27 AGAROSE	0	3 (AGAROSE)	4	24	0	3
Hp	7	3	4-20 (STEP GRAD)- 4-22 (CONT GRAD)	3 (STEP GRAD)	2 (CONT GRAD)	19	22	1	0

BECKMAN

CONFIRMATION TESTS

STAIN ID	CELLULOSE ACETATE						ACRYLAMIDE	
	EAP	PGM	EsD	AK	ADA	Gc	Hp	GLO-I
5	BA	2-1	1	1	1	-	2-1	
6	B	1	1	1	1	2-1	2-1	
7	CB	1	1	1	1	1	?	
8	BA	1	2-1	1	1	1	2-1	
12	B	1	2-1	1	2-1	-	2-2	
13	B	2-1	1	1	2-1	-	2-1	
14	BA	1	1	1	2-1	-	2-1	
15	B	1	1	1	2-1	-	1-1	
STANDARDS USED	AA	1-1	1-1	1-1	2-1	2-1	2-1	
	BA	2-2	2-2	-	1-1	2-2	2-2	
	BB	2-1	2-1	-	-	1-1	-	
	CB	-	-	-	-	-	-	
	CA	-	-	-	-	-	-	

BECKMAN

CONFIRMATION TESTS

STAIN ID	← STARCH THIN LAYER → EAP	PGM	EsD	AK	ADA	Gc	Hp	GLO-I
5	BA	2-1	1	1				2
6	B	1	1	1				2-1
7	CB	1	1	1				2-1
8	BA	1	2-1	1				2
12	B	1	2-1	1				2-1
13	B	2-1	1	1				2
14	BA	1	1	1				2-1
15	B	1	1	1				2-1
STANDARDS USED	BA	1-1						2

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RATIONALE FOR SELECTION OF STARCH/AGAROSE SUBSTRATE

BLIND TRIALS

DEMONSTRATED NEED FOR MORE THAN ONE TYPE OF SUBSTRATE: CAM, STARCH, ACRYLAMIDE STILL UNDER INVESTIGATION.

REVIEW OF SYSTEM DEFINITION SUMMARY

STARCH GEL SHOWN AS APPLICABLE TO LARGEST NUMBER OF CONSTITUENTS.

MULTISYSTEM ANALYSES REQUIREMENTS

THREE SUBSTRATE LIMITATION IN STATEMENT OF WORK. APPEARS MOST LIKELY TO BE MET WITH STARCH/AGAROSE.

SUMMARY OF SYSTEM DEFINITION

EQUIVALENT	STARCH GEL	CAM	ACRYLAMIDE
EsD	GLD 1	Ge	Hp
PGM	EAP		
Ak	ADA		

YIELDS UNAMBIGUOUS RESULTS

CAN BE DONE WITH LESS THAN 5 MANHOURS OF EFFORT IN 18 HOURS

ACHIEVE 1 IN 200 PROBABILITY OF DISCRIMINATION

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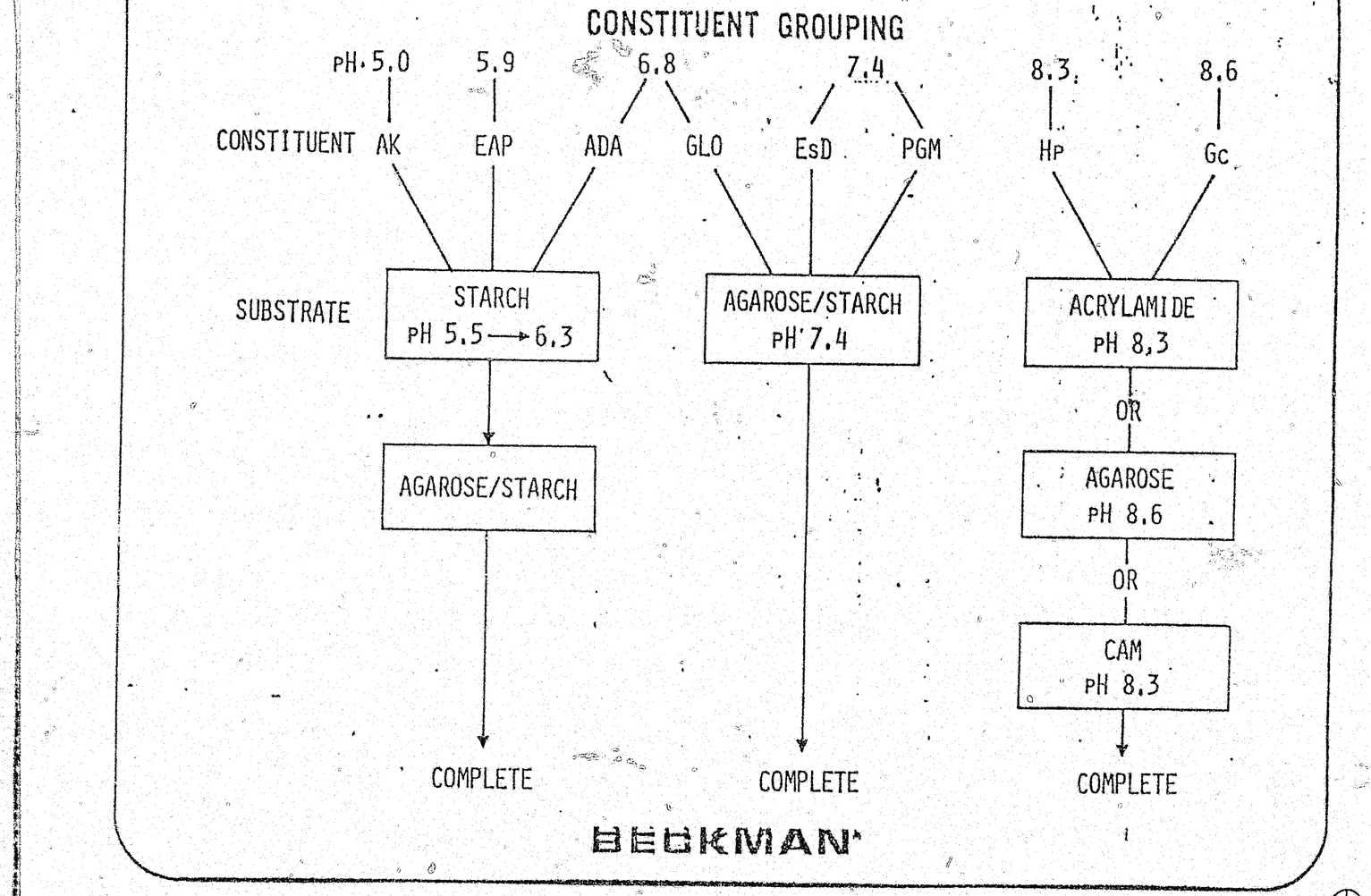
CONSTITUENT GROUPING
pH 5.0
5.9
6.8
7.4
8.3
8.6
CONSTITUENT
AK
EAP
ADA
GLO
EsD
PGM
Hp
Gc
SUBSTRATE
STARCH
pH 5.5 → 6.3
AGAROSE/STARCH
pH 7.4
ACRYLAMIDE
pH 8.3
OR
AGAROSE
pH 8.6
OR
CAM
pH 8.3
AGAROSE/STARCH
COMPLETE
COMPLETE
COMPLETE
BECKMAN

SIMULTANEOUS ANALYSIS DEVELOPMENT

GROUPS TESTED:

1. PGM, EsD, GLO-I
2. EAP, AK, ADA
3. Hp, Gc
4. PGM, EsD, AK

SUBSTRATES USED:

1. GROUP I – STARCH/AGAROSE
2. GROUP II – STARCH
3. GROUP III – ACRYLAMIDE, AGAROSE
4. GROUP IV – CELLULOSE ACETATE

NUMBER OF EXPERIMENTAL RUNS MADE:

1. GROUP I – 65
2. GROUP II – 47
3. GROUP III – 32

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SYSTEM DEVELOPMENT PROGRESS

GROUP I: PGM, EsD, GLOI

- SUBSTRATE -- 1% AGAROSE WITH 2% STARCH
- 4-WEEK-OLD STAINS HAVE BEEN CORRECTLY TYPED
- RUNNING TIME REDUCED FROM 4 HOURS (ON STARCH) TO 2 TO 3 HOURS
- IMPROVED SEPARATION OF GLO AND EsD
- SEPARATION DISTANCE IS 6 TO 7 INCHES.
- CONTINUE DEVELOPMENT TO
  - DETERMINE LIMITS FOR AMOUNT OF STARCH
  - OPTIMIZE GLO REACTION MIXTURE
  - OPTIMIZE EsD REACTION BUFFER
- HAVE HIGH CONFIDENCE FOR PRACTICAL SYSTEM

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SYSTEM DEVELOPMENT PROGRESS (CONT)

GROUP II: EAP, AK, ADA

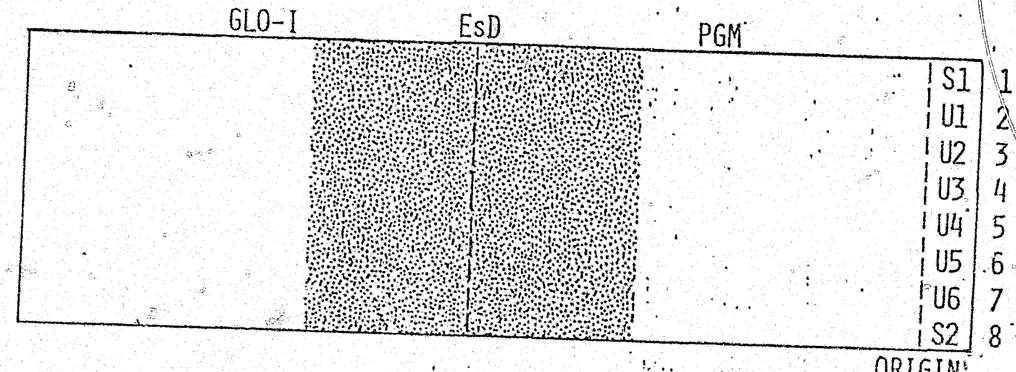
- SUBSTRATE – STARCH NOW, EVENTUALLY AGAROSE/STARCH OR AGAROSE
- SIGNIFICANT IMPROVEMENT IN EAP PHENOTYPES
- SOME IMPROVEMENT IN AK PHENOTYPES
- ADA SEEMS TO REQUIRE SLOW ELECTROPHORESIS TO OBTAIN GOOD REACTION
- AK AND EAP TEND TO OVERLAP: MAY MISS AK 3-1 VARIANT
- INDIVIDUAL ENZYMES HAVE DIFFERENT SENSITIVES TO REDUCING AGENTS
- SEPARATION DISTANCE IS 6 TO 7 INCHES
- EAP AND AK ARE ACCEPTABLE NOW: HAVE GOOD PROBABILITY OF INCLUDING ADA

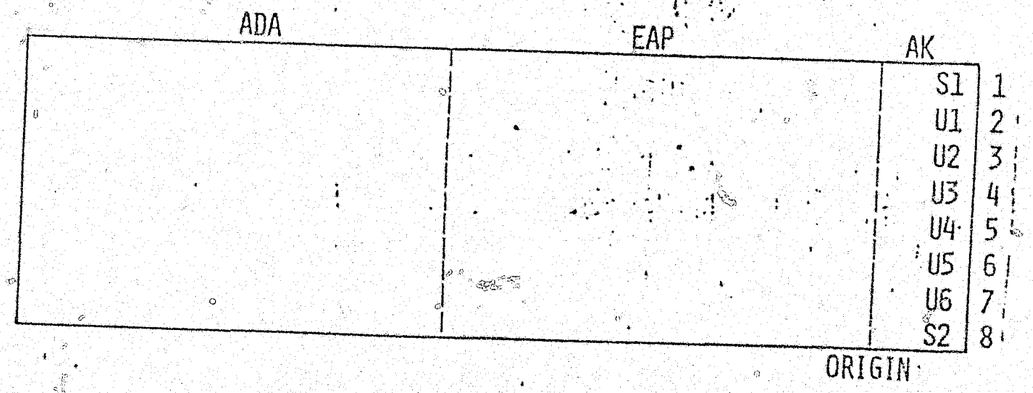
SYSTEM DEVELOPMENT PROGRESS (CONT)

GROUP III: Hp, Gc

- THESE CONSTITUENTS ARE THE MOST DIFFICULT TO COMBINE, EVEN THOUGH BOTH ARE PROTEINS.
- ANTIGEN-ANTIBODY CROSSED ELECTROPHORESIS SEPARATES Gc WELL, BUT ONLY MARGINALLY FOR Hp.
- ACRYLAMIDE SUBSTRATE WITH Hp DETECTION WITH O-TOLIDINE AND Gc BY IMMUNOFIXATION SEEMS MORE PRACTICAL THAN AACE.
- WITH STAINS ON ACRYLAMIDE, EXCESS Hb TENDS TO MASK THE VARIANT BANDING.
- THIS GROUP IS STILL CONSIDERED POSSIBLE, BUT WILL REQUIRE MUCH MORE WORK. A DECISION AS TO ITS PRACTICALITY WILL BE MADE NO LATER THAN NOVEMBER 1.

SERIAL SEPARATION





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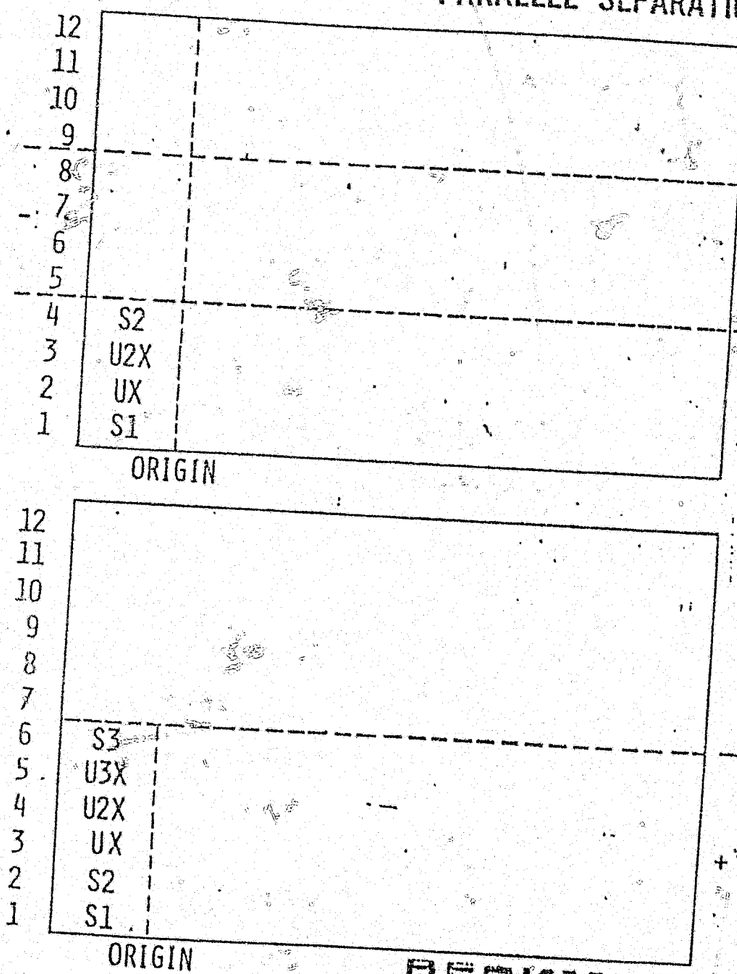
PARALLEL SEPARATION



SYSTEM I

PGM

EsD

GLO-I

SYSTEM II

EAP

ADA

AK

SYSTEM III.

H_P

Gc

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SEQUENTIAL TESTING - SINGLE CELL

ORDER OF ANALYSIS	SUBSTRATE	HANDS ON (MIN)	ELAPSED TIME (MIN)
SAMPLE PREPARATION	CAM	15	15
PGM	CAM	15	75
EsD	CAM	15	60
AK-ADA-EAP (SIMULTANEOUS)	CAM	25	60
GLO-I	ACRYLAMIDE GEL	30	150
Hp-Gc (SIMULTANEOUS)	ACRYLAMIDE GEL	35	120
TOTAL TIME (MINUTES)		135	480
		(2 HRS 15 MIN)	(6 HRS)

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EQUIPMENT SELECTION

COST LIMITATION – $6000 CAPITAL INVESTMENT

TRADEOFFS INVOLVED IN EQUIPMENT SELECTION

1. SERIAL SEPARATION VS PARALLEL
2. SIMULTANEOUS ANALYSES VS REPEATED SINGLE CELL

POSSIBLE CHOICES OF EQUIPMENT

1. MODIFIED MICROZONE
2. NANOPHOR
3. SHANDON
4. MULTICELL
5. MULTIPHOR – LKB INSTRUMENTS

FEASIBILITY TEST PLAN

SOW REQUIREMENTS – CRITERIA – ACCURACY
DEGREE OF INDIVIDUALIZATION
RAPIDITY
COSTS
CLARITY OF READING

ACCURACY – ACCURATELY IDENTIFY THE CONSTITUENTS IN A MINIMUM OF 90% OF THE UNKNOWN STAINS

DEGREE OF INDIVIDUALIZATION – 1 IN 200

RAPIDITY – 5 MANHOURS EFFORT IN 24-HOUR PERIOD PER STAIN

COSTS – NOT TO EXCEED $25 PER ANALYSIS

CLARITY OF READING – SUBJECTIVE REQUIREMENT TO BE DEFINED

NUMBER OF STAINS)
NUMBER OF TESTS) – MANHOUR LIMITATION--64 HOURS

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EQUIPMENT AVAILABILITY

1. MODIFIED MICROZONE – NEEDS COOLING PLATE
2. NANOPHOR – NOW LICENSED FOR MANUFACTURE
3. SHANDON – COMMERCIALLY AVAILABLE
4. MULTICELL – BECKMAN HAS SCHEDULED A PRODUCTION RUN
5. MULTIPHOR – COMMERCIALLY AVAILABLE – EXPENSIVE

END