FINAL REPORT

PROTOTYPE SLIDE STAINER

Contract NAS9-11929

August 1971

Prepared for:

National Aeronautics and Space Administration
Manned Spacecraft Center
Operations Equipment Development Branch
Houston, Texas

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National Aeronautics & Space Administration
Manned Spacecraft Center
Operations Equipment Development Branch
Houston, Texas 77058

Attention: Mr. J. L. Day, Mail Code DE52

Subject: Final Report
Development of Prototype Slide Stainer
for Period June 16 through August 31, 1971

Reference: Contract No. NAS 9-11929

Gentlemen:

During the period covered by this final report, the Advanced Technology Operations of Beckman Instruments, Inc. has designed, fabricated, and tested a prototype slide stainer. This letter and its attachments summarize the results of the contract. Attachments to this letter are as follows:

A. Top assembly drawing.

B. Verification Test Report, including Test Plan and Procedure.

C. Operating Procedures for Prototype Slide Stainer.

D. Materials and Parts List for Prototype Slide Stainer.

E. Reagent Compatibility and Stability Study.

THE DEVELOPMENT OF A PROTOTYPE SLIDE STAINER

The Objective

To design and fabricate a functional prototype of a slide staining system

capable of performing both one-component Wright's staining of blood smears and eight-step Gram staining of heat fixed slides of micro-organisms.

The Approach

Attention was given to liquid containment, waste handling, absence of contamination from previous staining, and stability of the staining reagents. The unit to be self-contained and capable of independent operation.

The Environment

The design objectives of this module were that it operate under one- or zero-g conditions and be compatible with Skylab A environmental conditions.

Primary Performance Objectives

The slide stainer module to be designed to perform the required slide stainer operations in a zero to one-g environment without contamination of the cabin atmosphere. The unit to be designed and fabricated to be compatible with Skylab A environmental conditions. The unit to have design objective weight of 4 pounds including expendables and maximum dimensions of 4.3 x 5.3 x 9.2 inches. The slide stainer shall have the capability of staining 48 Gram stains and 16 Wright stains, mixed intermittently.

Secondary Performance Characteristics

Slide Holder

The slide holder to be designed to confine the slide in such a manner as to allow reagents and rinse solutions to pass over the area to be stained without escaping to the cabin atmosphere.

Visual Indication

The slide to be mounted to allow visual indication that the various stains have covered the area of interest, and that the stains have been adequately flushed before the slide is removed.

Liquid Handling

The various stains and reagents to be confined and handled in a manner that prevents leakage and dissemination of liquids or vapors into the cabin.

Liquid Waste

The liquid waste from the staining procedure to be collected in a disposable plastic container. The volume of the reservoir to be adequate to contain all fluids and air flushes generated during the staining of six slides. The container to be designed to prevent the contents from spilling during disposal.

Operability, General Design Goals

Maintainability

The equipment to be designed to provide accessibility consistent with efficient testing, service, and maintenance during all phases of testing and operational activities. When the components become inoperable, they can be returned to operation within a time period as short as practicable.

Useful Life

The equipment to be designed to have a useful life of at least 2 years except for expendable equipment. Resupply and replacement or substitute components will be possible. Maintenance is an acceptable way of obtaining a useful life.

Shelf Life

The equipment, exclusive of reagents, to have a minimum shelf life of 3 years under normal warehouse conditions. Any limited-calendar life items to be identified and replacement assured, when required, by the station operating procedures. The desired shelf life of reagents is 9 months. Tests shall be performed to project actual shelf life.

Liquid Waste

The liquid waste from the staining procedure is collected in a disposable plastic container. The volume of the reservoir is adequate to contain all

fluid and air flushes generated during the staining of six slides. The container is designed to prevent the contents from spilling during disposal.

Operability, General Design Goals

Maintainability

The equipment is designed to provide accessibility consistent with efficient testing and operational activities. When the components become inoperable, they can be returned to operation within a very short time period.

Useful Life

The equipment is designed for a useful life of at least 2 years except for expendable equipment. Resupply and replacement or substitute components is possible. Maintenance is an acceptable way of obtaining a useful life.

Shelf Life

The equipment, exclusive of reagents, is designed to have a minimum shelf life of 3 years under normal warehouse conditions. Limited-calendar life items are identified and replacement will be assured by the station operating procedures. The shelf life of reagents is 9 months or more. Tests were performed to project an estimated actual shelf life.

Operating Life

Limited operating life items to be identified and on-time replacement, recalibration, or adjustments assured, when required, for the station operating procedures.

Environment

The equipment and its components to withstand the Skylab A environmental conditions.

The design concept of the prototype slide stainer is shown in the block diagram of Figure 1. Reagents are stored and dispensed from modified syringes fitted

with a screw drive for the plunger. The tip of each syringe is fitted with a cap holding a rubber septum. In use, the syringe tips are pushed over a needle mounted in a manifold and connected to a reagent selector valve. The needles penetrate the septum of each syringe and permit the liquid contents to be dispensed.

The common output of the selector valve is connected to a manifold which connects the valve with a common line leading to a staining chamber. Air and water for flushing are also connected into the manifold.

The staining chamber cover permits the positioning of a 1" x 3" microscopic slide over an "O" ring. Closing the chamber cover forces the slide onto the "O" ring forming a very small volume circular cavity between the surface of the glass slide and the bottom of the chamber. Small entrance and exit ports in the base of the chamber allows the flow of fluids through the formed cavity. Manually operated plunger type pumps force flushing water and air through the cavity to staining solutions.

On June 29, 1971, an informal Detail Design Review on the Slide Stainer was held at Beckman Instruments.

The design concept and the detail drawings were examined and discussed. A breadboard unit was used to demonstrate that the designed hardware would produce acceptable Gram Stains of micro-organisms and Wright Stained blood smears.

The breadboard contained and provided for the demonstration of the following essential components:

Reagent dispenser
Reagent selector valve
Staining chamber
Water and air dispenser
Waste collection method

During the demonstration of the breadboard, slides of B. subtilis (Gram Positive) and S. marcesens (Gram Negative) were stained followed by fixing and staining a blood smear with a one component Wright Stain. These preparations were adjudged to be satisfactory.

A discussion on the properties of materials of construction, such as that used for the stainer housing and reagent dispenser revealed that metals, i.e., aluminum or stainless were preferred over plastics such as Lexan. The extra weight of the metals was considered a less serious problem than the general tendency of plastics to be a source of combustible material.

Also during the discussion on weight tradeoffs, the use of ship's water was explored. Mr. Day objected to the use of ship's water on the basis that its use would require the astronauts to perform certain operations best performed on earth and all for the savings of one pound of water to be used over three missions.

The original design concept provided five reagent dispensers plus one dispenser for water rinse through the selector valve. During the breadboard evaluation of the system the sixth dispenser for water was found unnecessary. However, it was found that a sixth reagent dispenser containing absolute alcohol was necessary in order to flush out the slight amount of residual water in the staining chamber and common flow lines and also to fix the blood smear. Without the alcohol flushing of the system and fixation of the blood smear, the red cells are generally distorted and many of the cells appear to have small oil droplets superimposed upon them.

The laboratory method of fixing blood smears prior to staining utilizes methyl alcohol. Since this material is toxic to humans, its use in a manned spacecraft is to be avoided. In laboratory tests ethyl alcohol has been substituted for methyl alcohol in the blood staining method and found equally satisfactory. Therefore, the slide stainer will use absolute ethyl alcohol for dehydration.

Two minor changes in the design were suggested during the DDR. These are:

1. A permanent window be placed in the staining chamber cover to protect the small liquid ports from contamination. This would eliminate the need for a dummy slide to be kept in the chamber during non-use. Over long periods of time such a dummy slide would tend to flatten the "O" ring seal in the chamber and could result in a faulty seal.

2. A detent stop to be added to each reagent dispenser to indicate one full turn of the dispenser knob.

These two design changes have been made in the appropriate places.

On July 9, Mr. J. Walsh of Beckman met with Mr. N. Belasco and Dr. S. Poole of NASA at Lockheed Missile and Space Company, Sunnyvale, California.

The design of the slide stainer was again discussed. One design change was suggested. That being the addition of a key system on the reagent dispensers so that it would be impossible to place them in the wrong position. This change has been seriously considered, however, to incorporate such a change would require a complete redesign of the storage and locking mechanism as well as scrapping certain parts of the reagent dispensers which have been made. In view of the numbering system to be applied to the syringes and cavities, it is felt that the keys are not an absolute requirement for the prototype unit.

Prior to the start of the program, reagent stability tests and reagent compatibility tests with syringe materials were initiated. All glass syringes were found to freeze within two days and also leak badly. Plastic syringes with rubber tipped plunger absorbed the iodine from the Gram's iodine reagent. In addition, after several weeks at 37°C the rubber tips harden, swell or become stuck to the plastic barrel. The problem was solved with glass barrel syringes with TEFLON tipped plungers. During the reporting period the reagent stability compatibility tests have continued. After 36 days storage in the above described syringes, the Gram's iodine reagent has retained over 70% of its extractable iodine. Reagents stored for 36 days in the syringes at 37°C yielded gram stains of gram-positive and gram-negative organisms comparable with fresh reagents. A discussion of the compatibility and reagent stability problem is given in Attachment E.

An absorbent supplied by NASA for use in the waste bag which was labeled "Piddle-Pak" was evaluated along with a compressed sponge sold for cleaning soldering irons. The compressed sponge originally measuring 2" x 3" x 1/16" absorbed 25 ml of water in about 7 minutes while the Piddle-Pak material originally measuring 5 times greater in size absorbed only 10 ml of water in about 12 minutes. The sponge has been identified as a special pure cellulose acetate.

A mechanical analysis of the front door, which allows access to the waste bay storage, flushing valve assembly and water supply bag indicated that hinging the door on the right hand side, adjacent to the water and air flushing assembly, created a very weak mounting easily damaged. This was corrected by hinging the door along the bottom. A 3/4 inch hinge was replaced by a 5-1/2 inch hinge, thus making the door mounting much sturdier.

The design of the slide stainer requires plastic bags for water storage and waste collection. It is intended that the flight version of the Slide Stainer will be supplied with NASA approved and qualified plastic bags and from a composite material designed as SLP4. Because of the short time schedule allotted to the slide stainer prototype program, the SLP4 bags were not available. Therefore, for the prototype, suitably modified commercially available polyvinylchloride bags will be supplied. These bags will be similar in size only to those proposed for flight units of the stainer and will suffice for the prototype system. We have purchased heavy vinyl bags manufactured for blood transfusion purposes and have modified these. Heat sealing these heavy vinyl bags was accomplished by an outside vendor.

A significant improvement over the originally intended labeling of reagent dispensers and positions of the selector valve was accomplished by labeling the cover plate of the selector valve and the knurled knobs of the dispenser with color coded plastic discs which also contained a letter-number combination.

Although not a specified requirement, consideration was given to a safety valve which would stop the flow of fluid to the staining chamber in the absence of a slide in the chamber. Referring to Figure 1, it can be seen that such a safety valve would be attached to the liquid flow system at point X.

Numerous safety valve designs were studied and a valve design was finally selected for fabrication. The fabricated valve was found to require too large a volume, doubling the amount of reagents needed for a single stain.

During the preparation of detailed shop drawings, an analysis of the safety valve requirements revealed that in the worst case the reagent selector valve could be turned to a given reagent and without a slide in the chamber the knob of the reagent dispenser given one full turn. Since the safety valve could essentially block the flow of fluid to the staining chamber, a large hydrostatic pressure could be built up in the system. Such a pressure could cause a serious leak or rupture a connecting tube. The design was changed such that when a slide is not in the chamber any fluid accidentally being injected into the common flow lines would be directed to a safety waste bag which would always be connected in the system. Since one turn of a reagent dispenser now will inject 0.34 ml into the system, a safety waste bag of 2.5 ml would accommodate seven accidental occurrences.

After fabrication, the second safety valve was installed in the prototype unit and evaluated. This valve restricted the flow of reagents to the chamber and was very difficult to seal properly. Since time was limited and the two valves fabricated did not meet the requirements, a safety valve was not included in the prototype unit.

During the final assembly, testing, and also during the Verification Testing, a few difficulties or failures were noted. These were:

- The flushing valve located on the inside of the front access door has small Teflon tubing leading to it. Repeated bending and pulling on one of the lines resulted in a leak close to the valve. The valve and tubing was replaced. Locating the valve inside the right side access door with one flexible line leading to the flushing probe should eliminate this problem.
- The successful operation of the water drop dispenser requires that the fluid lines leading to the plunger pump and the drop port be primed. Priming of the lines has been found to be difficult at times.

Squeezing the water supply bag has been found helpful. However, a better method for dispensing the water drop would be a small version of the reagent dispenser syringes.

- Initially the glass barrel of the syringe was cemented in the dispenser housing using a silicone RTV material. It was thought that the soft rubber would hold the barrel in place and act as a cushion against vibration and shock. It was later found that the RTV material did not cure completely in the confined space. The use of filled epoxy cement for this application appears to have solved the problem.
- The stainless steel needles mounted in the manifold block were cemented in place with a small amount of epoxy cement. During use these needles are subjected to a backward pressure whenever the septum of the reagent dispenser is pushed onto the needle. Recementing the needles with a larger amount of epoxy has temporarily solved the problem. A better method of mounting the needles would be to braze them to a metal plate then attach the mounting block to the plate.

The completed prototype slide stainer is shown in Figures 2, 3 and 4. Major components and special features are identified.

The staining chamber accommodates standard 1" x 3" microscopic slides. However, since the actual area of the slide exposed to the staining reagents is limited, the slides must be prepared accordingly. The area of a slide which can be stained is limited to a 3/4" circle in the center of the slide.

Experimentation has shown that when making blood smears for the slide stainer the drop of blood should be placed at the very end of the slide in order to bring the feathered leading edge of the smear into the center of the slide.

Micro-organism slide preparation made from culture plates are best made as follows: Approximately 20 µl of water is placed in the center of the slide with the drop dispenser of the stainer. A sterile needle is then used to transfer a small quantity of a plate colony to the water drop. The material is then mixed with the water drop to form an almost clear suspension spread over an area about 1/2" in diameter.

The verification testing has demonstrated that the slide stainer has met the requirements specified in section 3.0 of the Work Statement with the exception of the weight design goal. The verification test report is found in Attachment B.

The slide stainer is expected to have a useful life in excess of two years. Replacement of septums in the reagent dispensers, flushing probe and waste port has been provided for. An occasional replacement of the "O" ring in the staining chamber may be necessary.

The normal shelf-life exclusive of reagents of the prototype slide stainer under normal warehouse conditions is expected to be greater than 3 years. The shelf life of the reagents with the exception of Gram's iodine can be confidently predicted to be in excess of 1 year. The shelf life of the Gram's iodine reagent has been projected to be approximately 9 months based on limited laboratory tests. Refer to Attachment E for a report on the stability testing of the reagents.

The only limited operating life items that can be identified at this time are the waste collection bags which have a predicted one time use and the septums in the reagent dispenser, the water bag connection and waste port. Provision for changing these have been provided.

Very truly yours,

BECKMAN INSTRUMENTS, INC.

John L. Brady

John L. Brady
Project Engineer
Advanced Technology Operations

JLB:jg
Attachments

ATTACHMENT B

VERIFICATION TEST REPORT

for

PROTOTYPE SLIDE STAINER

Contract NAS 9-11929

August 1971

Prepared for:

National Aeronautics and Space Administration
Manned Spacecraft Center
Operations Equipment Development Branch
Houston, Texas

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1.0 INTRODUCTION

A verification test was undertaken to demonstrate that the prototype slide stainer fulfilled the requirements, both primary and secondary, as defined in the contract specifications. With the exception of the 4 pound weight limit, all requirements were met by the prototype. The capability of staining both heat fixed micro-organism slide preparations and blood slide preparations in a self-contained unit that provides for liquid containment and capable of operating in a Skylab environment has been met. Weight reduction to meet flight hardware requirements can be accomplished by material substitution and/or component modification.

The verification test was conducted at Beckman Instruments' facility in Fullerton, California, on August 26th and 27th. Mr. J. Day and Dr. C. Ferguson of NASA MSC witnessed the testing. Dr. Ferguson was present only on the 26th.

2.0 VERIFICATION TEST

2.1 Scope

The Prototype Slide Stainer shall be a self-contained system capable of performing both an eight-step Gram Stain of micro-organisms and a one-component Wright's stain of blood snears. The verification test shall demonstrate the performance characteristics described below.

3.0 PERFORMANCE CHARACTERISTICS

3.1 Primary

3.1.1 The unit shall have the capability of staining both heat fixed micro-organism slide preparations with an eight-step Gram stain and blood smears with a one-component Wright's stain, mixed intermittently. A capability of 48 Gram stains and 16 Wright stains is required.

3.1.2 The Gram stained micro-organism preparations and Wright-stained blood smears to be equivalent to similar preparations stained by standard laboratory methods.

3.1.3 The stainer shall be capable of operating in a zero to 1 g environment without contamination of the cabin atmosphere.

3.1.4 Maximum dimensions to be 4.3 x 5.3 x 9.2 inches.

3.1.5 Maximum weight of 4 pounds.

3.2 Secondary

3.2.1 The slide holder shall confine the slide in such a manner as to allow reagents and rinse solutions to pass over the area to be stained without escaping to the cabin atmosphere.

3.2.2 The slide holder shall allow visual indication that the various stains have covered the area of interest, and that stains have been adequately flushed before slide removal.

3.2.3 The various stains and reagents shall be confined and handled in a manner that prevents leakage and dissemination of liquids and vapors into the atmosphere.

3.2.4 The liquid waste from the staining procedure will be collected in a disposable plastic container. The volume of each waste container will be adequate to contain all fluid and air flushes during the staining of six slides. The waste container shall be designed to prevent the contents from spilling during disposal.

3.2.4.1 The waste containers shall contain a liquid absorbent capable of absorbing the liquid waste from staining six slides.

3.2.4.2 The waste containers shall be stored within the staining unit and be easily accessible.

3.2.5 A means of dispensing a drop of water for slide preparation shall be provided.

3.2.6 The reagent and stain storage and dispensing containers to be of a material(s) which will not affect the normal stability of stains or reagents.

3.2.7 Reagent and stain transport system to have minimum contamination from previous staining.

3.2.8 Easy resupply of reagents and wash water.

3.2.9 A means of flushing entire system when required.

3.2.10 A hold-down connection shall be provided in the bottom of the unit.

3.2.11 Reagent and stain dispensers and valve positions shall be positively identified.

3.2.12 Accessibility for maintenance to provide required useful life and shelf life.

4.0 VERIFICATION TEST PROCEDURE

4.1 Physical Inspection

4.1.1 A physical inspection was conducted to demonstrate the requirements listed in Table 1. The test results are given in Paragraph 5.1.

4.2 Staining Demonstration

4.2.1 The staining of micro-organism slide preparations and blood smears was conducted to demonstrate the ability of the slide stainer to meet the remainder of the requirements not covered in Section 4.1. The results are given in Paragraph 5.2.2.

TABLE 1

Requirement	Inspection Method	Reference Paragraph
Dimensions	Measurement	3.1.4
Weight	Measurement	3.1.5
Visibility of Slide	Visual	3.2.2
Storage	Visual	3.2.4.2
Self-containment	Visual & analysis	2.1
Resupply	Demonstration	3.2.8
Hold-down	Visual	3.2.10
Labeling	Visual	3.2.11
Accessibility	Demonstration	3.2.12

4.2.1.1 The staining demonstration consisted of staining of four Gram-positive and three Gram-negative organisms, and one which showed both positive and negative Gram staining characteristics. These organisms were supplied by NASA as lyophilized preparations several weeks prior to the testing. The week prior to the testing, the lyophilized preparations were cultured in a nutrient broth then on casein soy agar plates. After approximately sixteen hours growth, slides were prepared from each plate.

Blood smears prepared the evening before the test were utilized.

In addition, four slides prepared from six day old stock cultures of S. Aureus and E. coli were stained with the slide stainer. These cultures were supplied by Dr. Ferguson on the testing date.

4.2.1.2 Zero capability was demonstrated by analysis.

4.2.1.3 Blood smears were stained with a one-component Wright's stain intermittently between the micro-organism slides.

4.2.1.4 To demonstrate the adequate staining of micro-organisms and blood smears by the slide stainer, duplicate micro-organism slides and blood smears were stained concurrently by a standard laboratory method and a comparison made.

4.2.1.5 The integrity of fluid connections was established by examination after the staining demonstration.

4.2.1.6 The safety features of the slide stainer, reagent containers and waste containers were demonstrated during and following the staining procedure.

5.0 VERIFICATION TEST RESULTS

5.1 Physical Inspection

DIMENSIONS (Para. 3.1.4)

Length: 9.192 inches, within limits.

Height: 4.306 inches, 0.006 inches over limit on high spot at one corner.

Width: 5.312 inches, 0.012 inches over limit on high spot of one reagent dispenser.

Dimensions acceptable.

WEIGHT (Para. 3.1.5) Weight including 130 ml water, 5 waste bags, and 6 reagent dispensers with approximately 12 ml each: 7 pounds.

Weight 3 pounds over design objective.

VISIBILITY OF SLIDE (Para. 3.2.2)

Slide visible through window in staining chamber cover. Acceptable.

STORAGE OF WASTE CONTAINERS (Para. 3.2.4.2)

A storage compartment behind the front access door will accommodate 4 waste bags; storage compartment behind the right hand access door will accommodate 2 waste bags. Acceptable.

SELF-CONTAINMENT (Para. 2.1)

Self-containment demonstrated by performing both Gram stains and one-component Wright's stains with the stainer system. All supplies necessary for the staining procedures, such as stains, flushing water and waste collecting bags were contained within the slide stainer. However, during the staining procedure, a waste collecting bag is connected to an external waste port. Self-containment acceptable.

RESUPPLY (Para. 3.2.8)

The ease of resupply was demonstrated by exchanging used reagent dispensers with full dispensers. The used dispensers were rotated counter-clockwise approximately one-quarter turn then pulled out of the stainer housing. Full dispensers were then replaced in the unit by the reverse process. The water supply bag was exchanged with a full one by opening the front access door, pulling out the water supply bag container and disconnecting the water bag from the unit by a quarter turn counter-clockwise of the quick-disconnect liquid coupling then pulling apart. A full water bag was then inserted in the metal water supply bag container, the liquid couplings pushed together and locked with a quarter turn clockwise. The water supply bag container was then slid back into position and the access door closed. The waste collecting bags are resupplied by opening the front and side access doors and placing such bags in the storage areas provided. Resupply acceptable.

HOLD-DOWN (Para. 3.2.10)

A hold-down connection consisting of a 1/4-20 threaded insert has been provided in the bottom of the unit, approximately centered and one and one-half inches from the front. Acceptable.

LABELING (Para. 3.2.11)

Each of the reagent dispensers has been positively labeled with a letter and number and color coded. The reagent selector valve positions are also labeled with the same letter-number combinations and color coded. The front face of the slide stainer is labeled to show the position of the four reagent dispensers for the Gram stain and the position of the two Wright's stain reagent dispensers. The front and right side access doors identify that which is behind them. The water drop dispenser and the air, water, water drop plungers are identified. Two items which are not labeled are the knob of the reagent selector valve and the knob of the staining chamber cover. These are two minor omissions which are readily corrected. Labeling acceptable.

ACCESSIBILITY (Para. 3.2.12)

Accessibility for maintenance to provide required useful life and shelf life is provided by removal of the bottom plate which gives access to all the components requiring service except the air, water and drop plungers. The replacement of "O" rings and springs for these three plungers is accomplished by removal of the engraved name plate holding these plungers in position. Accessibility acceptable.

5.2 Performance and Staining Demonstration

5.2.1 Performance Results

During the staining of the micro-organism slide preparations and the blood slides, the following required performance characteristics were demonstrated:

- The ability of the slide holder to confine the slide in such a manner as to allow reagents and rinse solutions to pass over the area to be stained without escaping to the cabin atmosphere (Para. 3.2.1).
- The ability of the slide staining cavity to facilitate the flow of reagents without serious entrapment of bubbles during the staining periods.
- The ability of the slide staining system to adequately flush the stains from the chamber prior to slide removal (Para. 3.2.2).
- The confinement and handling of the stains and reagents in a manner that prevents leakage and dissemination of liquids and vapors into the cabin atmosphere (Para. 3.2.3).
- The collecting of liquid waste and flushing air in disposable plastic containers, the volume of each to be adequate to contain all fluid and air flushes resulting from four Gram stains and two Wright's stains (Para. 3.2.4).
- The waste containers to contain a liquid absorbent capable of absorbing the liquid resulting from the four Gram stains and two Wright's stains, and designed to prevent spillage of the contents during disposal. This accomplished by incorporating a check valve in the quick-disconnect liquid connector (Para. 3.2.4.1).

- The liquid transportation system was shown to exhibit a sufficiently low reagent residual that contamination from the previous staining did not adversely affect the following staining with the exception of the Wright's staining of blood smears. Prior to fixing blood smears with absolute alcohol the liquid transportation system, which contains residual water from the previous staining, must be flushed out with the absolute alcohol to remove the water. This flushing has been found necessary since during the fixing process any water which contacts the blood smear will wash away or distort the red blood cells present on the slide. The flushing procedure prior to staining of blood smears requires three extra steps which require less than 15 seconds and does not appear to be objectionable (Para. 3.2.7).

The water drop dispenser was shown to perform adequately after preliminary priming of the lines was accomplished (Para. 3.2.5).

The flushing system was demonstrated on the 26th after approximately 20 slides had been stained. Reagent dispenser G4 was removed from the slide stainer, the front access door was opened and the flushing probe removed, and a dummy slide was placed in the staining chamber. The flushing valve also inside the front access door was turned to the flushing position and the reagent selector valve turned to G4 position. The tip of the flushing probe, containing a rubber septum, was inserted into the G4 guide tube and pressed onto the needle at the far end. The water plunger was then pressed three times, at which time all the red color had been flushed from the liquid system into the waste bag. It was explained that flushing out of the remaining five reagent lines would be performed in a like manner (Para. 3.2.9).

5.2.2 Staining Demonstration Results

Blood smears and micro-organism slide preparations, as described in paragraph 4.2.1.1, were stained with the prototype slide stainer following the operating procedures developed for the unit. A number of similar preparations were stained using standard laboratory methods with reagents identical to those used in the prototype slide stainer.

Those blood smears which had been stained in the slide stainer were examined by both Dr. Ferguson and Mr. Day and found to be a good Wright's stain; the erythrocytes were a pink color, the granules of the neutrophils blue to lilac, the eosinophils red, and the cytoplasm of the monocytes a pale blue. The blood staining was judged acceptable.

The results of the staining of the micro-organism slide preparations are given in Table 2, Morphology and Staining Characteristics of Micro-organisms Supplied by NASA.

The staining demonstration showed that a minimum of 16 Gram stains and 8 Wright stains could be performed with the reagents and supplies contained within the slide stainer. Since the reagent dispensers, water supply bag and waste collecting bags are easily replaced within approximately ten minutes, the slide stainer has a capability in excess of that required (Para. 3.1.1).

TABLE 2. MORPHOLOGY AND STAINING CHARACTERISTICS OF NASA SUPPLIED MICRO-ORGANISMS

Identification	Morphology	Staining Characteristics
273	bacilli	gram-positive
274	bacilli	gram-negative
276	bacilli	gram-negative
278	diplococci	gram-negative
300	cocci	mixed gram-negative and gram-positive
301	cocci	gram-positive
302	cocci	gram-positive
308	cocci	gram-positive
S. Aureus	cocci	gram-positive
E. Coli	bacilli	gram-negative

During the staining demonstration an objection was raised, by those observing, that in the Gram staining procedure two full measures of alcohol, reagent G3, were used for decolorizing, which limited the total number of Gram stains per

reagent set to less than 16. To correct this problem, it was suggested that for each Gram stain decolorizing step the reagent dispenser G3 knob be rotated one-half turn instead of a full turn. Thus, only one-half the amount of reagent G3 would be required for each stain. This procedure was tried with micro-organism 273 and 301 and in each case the results were the same as those in Table 2 in which twice the amount of decolorizing alcohol, reagent G3, was used.

After Dr. Ferguson examined a few of the gram-positive staining micro-organism preparations which had been stained in the slide stainer, using 2 minutes staining time for the counter stain, reagent G4, he questioned the appearance of what looked like gram-negative micro-organisms and commented that such could confuse a non-trained observer. Dr. Ferguson suggested that the gram-negative appearing structures might be a result of too long a counterstaining time. An experiment was tried in which both a gram-positive and a gram-negative micro-organism were stained in the slide stainer using one minute counter-staining time with reagent G4. The gram-negative micro-organisms appeared the same as with the two minute staining time but the gram-positive preparation showed a reduced amount of the gram-negative appearing substance.

5.2.3 Staining Procedures Compatibility with Skylab Potable Water

The water requirements for 16 Gram stains and 8 Wright's stains plus that required for flushing the lines after a mission and a safety factor of 25% additional water totals 130 ml. Since one of the intended uses of the slide stainer will be on Skylab, a reduction in weight of the expendable supplies required for the stainer could be realized if the staining procedures could utilize ship's water for flushing. The Skylab potable water supply system is expected to supply water containing 36 ppm of potassium iodide and 6 ppm of iodine, as a means of sterilizing the water. The effect of these sterilizing agents on the staining procedures of the Gram stain and Wright's stain was unknown. Thus an experiment was conducted to test the staining procedures with flushing water simulating that which would be obtained from Skylab's potable water supply. Duplicate blood smears and gram-positive and gram-negative micro-organism slide preparations were made and stained. One set used distilled water for flushing and the other used water containing 36 ppm of potassium iodide and 6 ppm of iodine. Microscopic examination of both sets of slides revealed no detectable difference in the staining.

ATTACHMENT C

OPERATING PROCEDURES

for

PROTOTYPE SLIDE STAINER

Contract NAS 9-11929

August 1971

Prepared for:

National Aeronautics and Space Administration
Manned Spacecraft Center
Operations Equipment Development Branch
Houston, Texas

Beckman® INSTRUMENTS, INC.
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FULLERTON, CALIFORNIA • 92634

1.0 STORAGE

The prototype slide stainer can be stored with the reagent dispensers and water supply bag filled. During storage the reagent dispensers are held in a storage position with the black ring on the body of the dispenser visible. The water supply bag is not connected to the system during storage. Unused waste collecting bags, in their compressed folded condition, are stored in the two storage compartments.

2.0 STORAGE MODE TO STAINING MODE

The reagent dispensers are changed from storage mode to staining mode by rotating the body of the dispenser clockwise and pushing inward simultaneously until a stop is felt. The black ring on the body will no longer be visible and the dispenser will be held in position by a spring/pin and notch arrangement. With the two Wright's stain dispensers, W1 and W2, in the staining position, the front access door can now be opened. The water storage bag in its holder is now pulled out of the stainer unit and connection made to the liquid system by pushing together the two mating quick-disconnect fittings. A quarter turn clockwise locks the fittings together. The water storage assembly is then returned to its original position. Before closing the front access door, the flushing valve is set in the staining position and one of the waste collecting bags is removed and connected to the waste port on the right side of the unit. The fitting on the waste bag is pushed into the waste port connection and locked in position by a quarter turn clockwise. The waste bag is disconnected by the reverse procedure.

A dummy slide is next placed in the staining compartment. The water drop dispensing line and the water flushing lines require priming prior to operation. This is accomplished by partially removing the water supply assembly from the staining unit, gently squeezing the plastic water bag while pumping the

appropriate plunger located on the right front of the unit until water emerges from the water drop dispenser port and water is noticed to flow into the staining chamber. The water in the staining chamber is now flushed out by depressing the air plunger.

The fluid transfer lines between the reagent selector valve and the reagent dispensers must now be filled. With the dummy slide still in the staining chamber, the reagent selector valve is turned to the G1 (green) position and the knurled knob on the G1 (green) reagent dispenser is turned clockwise until a deep purple liquid is observed just flowing into the staining chamber. This requires one to two turns of the reagent dispenser's knurled knob. The fluid in the staining chamber is flushed out by depressing the air plunger once. The reagent selector is next turned to position G2 (yellow) and the same procedure followed with reagent dispenser G2 (yellow). All six reagent transfer lines are filled in the same manner. After the last reagent line, the sixth, has been filled the staining chamber is flushed out with water by depressing the water plunger then flushed out with air. The dummy slide is now removed and the chamber wiped dry with a tissue. The unit is ready for staining.

3.0 GRAM STAIN PROCEDURE

Step	Operation	Estimated Time (Seconds)	Volume (ml)
1.	Remove dummy slide from slide holder - cover of staining chamber if necessary.	10	
2.	Insert microbiological slide in slide holder/ cover of staining chamber - material side toward operator - close cover of chamber and secure.	15	
3.	Rotate reagent selector valve clockwise to G1 reagent (crystal violet) position (color code green).	5	
4.	Turn knurled knob of #1 reagent dispenser (color code green) clockwise 1 full turn.	5	0.35
5.	Stain	60	
6.	Depress air flush plunger slowly.		3.0

Step	Operation	Estimated Time (Seconds)	Volume (ml)
7.	Depress water flush plunger slowly.	5	1.0
8.	Depress air flush plunger slowly.		3.0
9.	Rotate reagent selector valve clockwise to G2 reagent (Lugol's iodine) position (color code yellow).	5	
10.	Turn knurled knob of G2 reagent dispenser (color code yellow) clockwise 1 full turn.	5	0.35
11.	Stain	60	
12.	Depress air flush plunger slowly.		3.0
13.	Depress water flush plunger slowly.		1.0
14.	Depress air flush plunger slowly.		3.0
15.	Rotate reagent selector valve clockwise to G3 reagent (alcohol) position (color code white).		
16.	Turn knurled knob of G3 reagent dispenser (color code white) clockwise 1/2 turn.		
17.	Decolorize	20	0.35
18.	Repeat Steps 16 and 17	20	
19.	Depress air flush plunger		3.0
20.	Depress water flush plunger	5	1.0
21.	Depress air flush plunger		3.0
22.	Rotate reagent selector valve clockwise to G4 reagent (Safranin) position (color code red).	5	
23.	Turn knurled knob of G4 reagent dispenser (color code red) clockwise 1 full turn.	5	0.35
24.	Counterstain	60	
25.	Depress air flush plunger slowly.		3.0
26.	Depress water flush plunger slowly.	5	1.0

Step	Operation	Estimated Time (Seconds)	Volume (ml)
27.	Depress air flush plunger.		3.0
28.	Remove slide from slide holder - blot on absorbent pad - place in incubator to dry.	60	
29.	Dry slide	?	
30.	Wipe staining area and "O" ring with absorbent tissue.	60	
	Time Total	415 sec (6.9 min)	
	Stain Total	1.4 ml	
	Water Total	4.0 ml	
	Air Total	24.0 ml	
	Volume Total 1 Slide	29.4 ml	
	Waste Bag Capacity-Air Plus Liquid	150 ml	
	Stained Slides per Waste Bag		
	Gram Stain	4 (29.5x4 = 118.0 ml)	
	Blood Stain	2 (14.4x2 = 28.8 ml)	
		(118 + 28.8 = 146.8 ml)	

Reagent dispenser capacity 12 ml approximately.

Reagent per stain 0.35 ml - 0.7 ml required for filling lines.

16 stains per mission 16 x 0.35 = 5.6 ml required.

Excess reagents 12 ml - (0.7 + 5.6 ml) = 5.7 ml or 100% safety factor.

STAINING UNIT TO STORE A MINIMUM OF 6 150 ml WASTE BAGS.

First bag used during filling of liquid transfer lines plus 3 Gram stains and 2 blood stains. Last waste bag used for 2 Gram stains and 3 blood stains plus water and air from flushing operations.

4.0 BLOOD SLIDE (WRIGHT'S) STAIN PROCEDURE

Step	Operation	Estimated Time (Seconds)	Volume (ml)
1.	With dummy slide in slide holder, rotate reagent selector valve to W1 reagent position (color code clear).	5	
2.	Turn knurled knob of W1 reagent dispenser (methyl alcohol) clockwise 1 turn.	5	0.35
3.	Depress air flush plunger slowly.	5	3.0
4.	Remove dummy slide from slide holder - cover of staining chamber.	10	
5.	Insert blood slide in slide holder/cover of staining chamber - blood side toward operator close cover of chamber and secure.	15	
6.	Again turn knurled knob of W1 reagent dispenser (color code clear) clockwise 1 turn.	3	0.35
7.	Fix blood smear	60	
8.	Depress air flush plunger slowly.	2	3.0
9.	Rotate reagent selector valve to W2 reagent (Wright's stain) position (color code blue).	5	
10.	Turn knurled knob of W2 reagent dispenser (color code blue) clockwise 1 turn.	5	0.35
11.	Stain	30	
12.	Repeat steps 10 and 11.	35	0.35
13.	Rotate reagent selector valve to position between W1 and W2.	5	
14.	Depress water flush plunger slowly.		1.0
15.	Wait	(60)	
16.	Depress air flush plunger twice	5	6.0
17.	Remove slide from slide holder - blot on absorbent pad - place in incubator to dry.	60	

Step	Operation	Estimated Time (Seconds)	Volume (ml)
18.	Dry slide	?	
19.	Wipe staining area and "O" ring with absorbent tissue.	60	
	Time Total	370 Seconds (6 min. 10 sec.)	
	Stain Total	1.4	
	Water Total	1.0	
	Air Total	12.0	
	Volume Total per Slide	14.4	

Reagent dispenser W2 (blood stain) total capacity approximately 12 ml or 16 blood stains per mission.

5.0 FLUSHING PROCEDURES

After use and prior to storage between missions, complete flushing of the liquid transport system has been recognized as a requirement of the system. This is accomplished as follows:

Step	Operation
1.	Place dummy slide in staining chamber.
2.	Attach waste bag to waste port on right side.
3.	Remove all reagent dispensers from the staining unit by rotating the body of each counter-clockwise while allowing the internal spring to push the dispenser part way out.
4.	Complete removal by pulling dispenser straight out.
5.	Open the front access door and remove the plastic flushing probe.
6.	Rotate the flushing valve handle, located in the lower portion of the door assembly, to the flushing position.

Step	Operation
7.	Rotate the reagent selector valve to G1 (green) position.
8.	Insert the flushing probe into the G1 guide tube and press the tip, containing the rubber septum, over the needle located at the far end of the guide tube.
9.	Depress the water plunger three times to flush out the G1 reagent line.
10.	Repeat steps 7 through 9, changing the position of the reagent selector valve and flushing probe to flush out the remaining five reagent lines.
11.	Remove the dummy slide from the staining chamber.

ATTACHMENT D

PARTS AND MATERIALS LIST

FOR

PROTOTYPE SLIDE STAINER

Contract NAS 9-11929

August 1971

Prepared for:

National Aeronautics and Space Administration
Manned Spacecraft Center
Operations Equipment Development Branch
Houston, Texas

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The following Parts and Materials list contains two material columns, one for the Prototype and one for an eventual flight unit. In many parts, the materials listed for the prototype can be substituted by other materials for the flight unit should a trade-off analysis indicate an advantage for either weight reduction or change to higher ignition temperature material.

SLIDE STAINER PARTS AND MATERIALS LIST

PART	MATERIAL	
	PROTOTYPE	FLIGHT UNIT
Housing, main body and bottom plate	Aluminum 6061 clear Anodized	Subject to Analysis
Reagent Selector Valve (1) (Chromatronics, Inc.)		
Body	Teflon	Teflon
Rotor	Kel-F	Kel-F
Connecting tubing	Teflon	Teflon/SS
"O" Ring	Buna-n	Buna-n
Shaft	SS	Subject to Analysis
Knob and Skirt	Aluminum	Subject to Analysis
Mounting Ring	Delrin	Delrin
Lubricant	Fluorocarbon grease	Fluorocarbon grease
Staining Chamber (1)		
Body	Polycarbonate	Subject to Analysis
Cover	Aluminum	Subject to Analysis
Knob, Sealing	Aluminum	Subject to Analysis
Knob, retain ring	SS	SS
Window	Plexiglass	Subject to Analysis
Slide Retaining Spring	Beryllium copper	SS
Washer	Teflon	Teflon
Screw	Steel	Subject to Analysis
"O" Ring	Buna-n	Buna-n
Flushing Valve (1) (Hamilton Co. Special)		
Body	Aluminum/Kel-F	Subject to Analysis
Rotor	Teflon	Teflon
Shaft & Knob	SS	Subject to Analysis
Tubing	Teflon	SS/Teflon
Adhesive	Epoxy	Epoxy
Flushing Probe (1)		
Body	Delrin	Subject to Analysis
Tubing	Teflon	Teflon
Septum	Silicone rubber	Silicone rubber
Check valve	Rubber?	Buna-n

	MATERIAL	
PART	PROTOTYPE	FLIGHT UNIT
Reagent Dispenser Guide Blocks & Mounting Blocks		
Block, Guide	Polycarbonate	Subject to Analysis
Block, Mounting	Polycarbonate	Subject to Analysis
Spring (6)	Steel	SS
Spring retainer (6)	Aluminum	Aluminum
Needle (6)	SS	SS
Adhesive	Epoxy	SS Braze
Keenserts (22) (KNCB 0440)	Steel	Steel
Reagent Dispenser, Basic Syringe (12) (Hamilton Co.)		
Barrel	Glass	Glass
Plunger	SS	Subject to Analysis
Plunger tip	Teflon	Teflon
Plunger ring	Brass	Subject to Analysis
Bushing	Delrin	Delrin
Body	Aluminum 6061	Subject to Analysis
Knob/Drive screw	Steel	Subject to Analysis
Pins	Steel	Steel
Ball-plunger	Steel	Steel
Retaining ring	Steel	Steel
Adhesive	#1 Silicone RTB	
	#2 Epoxy	Epoxy
Septum	Silicone Rubber/ Teflon	Silicone Rubber/ Teflon
Engraved Knob Insert	Colored Plexiglass	Subject to Analysis
Water Drop Dispenser Port		
Body block	Polycarbonate	Polycarbonate
Cover	SS	Aluminum
Cover Pad	Cotton felt	Subject to Analysis
Water, Air, and Drop Dispenser Assay		
Body	Polycarbonate	Subject to Analysis
Plunger	Delrin, black	Subject to Analysis
Springs	Steel	SS
"O" Rings	Buna-n ?	Buna-n
Lubricant	Fluorocarbon grease	Fluorocarbon grease
Keenserts	Steel	Steel
Cover & Retaining Plate	Aluminum	Subject to Analysis

PART	MATERIAL PROTOTYPE	FLIGHT UNIT
Front Access Door		
Door	Aluminum 6061	Subject to Analysis
Hinge	Aluminum	Subject to Analysis
Hinge Pin	Steel	Subject to Analysis
Knob and Catch	Steel	Subject to Analysis
Retaining Ring	Steel	Steel
Right Side Access Door		
Door	Aluminum	Subject to Analysis
Hinge	Aluminum	Subject to Analysis
Hinge Pin	Steel	Steel
Handle	Steel Wire	SS Wire
Snap Slide Fastener	Steel	Steel
Check Valves, Duckbill (17 Total)		
Housing #1	Aluminum	Subject to Analysis
Housing #2	Polycarbonate	Polycarbonate
Valve	Buna-n	Buna-n
Connecting Manifold (2)		
Body	Polycarbonate	Polycarbonate
Septum Removal Tool, (Water Bag & Waste Port)		
Tool	Steel	Subject to Analysis
Chain	Brass	Subject to Analysis
Tubing, Connecting	Teflon	Teflon/SS
Connectors, Tubing (38 sets)		
Nut	Delrin	Subject to Analysis
Washer #1	Steel	Subject to Analysis
Washer #2	Brass	Subject to Analysis
"O" Ring	Buna-n ?	Buna-n
Waste Port		
Body	Aluminum	Subject to Analysis
BNC Mod. Connectors	Brass/Nickel/Delrin	Subject to Analysis
Septum	Silicone Rubber	Silicone Rubber

PART	MATERIAL: PROTOTYPE	MATERIAL: FLIGHT UNIT
Water Storage Bags		
Bag	Polyvinylchloride Plastic	SLP-4
BNC Mod. Connectors	Brass/Nickel/Delrin	Subject to Analysis
Tubing	Polyvinylchloride	Subject to Analysis
Septum	Silicone Rubber/ Teflon	Silicone Rubber/ Teflon
Water Storage Bag Housing		
Housing	Aluminum	Subject to Analysis Need Questioned.
Waste Collecting Bags		
Bag	Polyvinylchloride Plastic	SLP-4
BNC Mod. Connectors	Brass/Nickel/Delrin	Subject to Analysis
Conn. Needle	SS	SS
Check Valve	Buna-n	Buna-n
Tubing	Polyvinylchloride Plastic	Subject to Analysis
Absorbent	Cellulose Sponge (Special)	Cellulose Sponge (Special)
Hold-Down Fitting		
Fitting	Steel	Steel

REAGENTS

Gram Stain

Reagent G1	Crystal Violet Crystal Violet 2 gm. Alcohol, Reag. 20 ml. Ammonium Oxalate 0.8 gm Water, Dist. 80 ml.	No Feasible Alternate
Reagent G2	Gram's Iodine Iodine 1 gm Pot. Iodide 2 gm Water, Dist. 300 ml	No Feasible Alternate
Reagent G3	Decolorizine Alcohol Ethanol SDA-3A 95% Isopropyl 5%	
Reagent G4	Counter Stain Safranin (2.5% in Alc) 10 ml Water, Dist. 100 ml	

Wright's Stain (Blood)

Reagent W1	Absolute Methanol	Absolute Ethanol
Reagent W2	Commercial Product[1] Wright's Stain Buffers Methanol 95%	No Feasible Alternate

Note: All stains aged 48 hours after mixing, then filtered.

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(1) Camco Quik Stain manufactured by Cambridge Chemical Products, Inc.

ATTACHMENT E

REAGENT COMPATIBILITY AND STABILITY STUDY

for

PROTOTYPE SLIDE STAINER

Contract NAS 9-11929

August 1971

Prepared for:

National Aeronautics and Space Administration
Manned Spacecraft Center
Operations Equipment Development Branch
Houston, Texas

Beckman® INSTRUMENTS, INC.
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1.0 INTRODUCTION

The stability of laboratory reagents has long been a subject of concern and much study. The literature contains much information on the stability of chemical reagents and analytical solutions, but much information is lacking on the stability of biological staining preparations. This lack of data on stains is especially noticeable in the area of storage or compatibility of the stains when in contact with many of the recently developed plastics.

The initial concept of a slide stainer for space application projected the use of lightweight plastics for many parts of such a slide stainer. The current prototype slide stainer development originally considered utilizing modified plastic syringes for both storage and dispensing purposes. However, lack of compatibility and stability data delayed the final configuration of the dual purpose containers which were to be both storage and dispensing units.

2.0 PRELIMINARY STUDIES

Four months prior to the awarding of the present slide stainer contract, the stability and compatibility of the staining reagents with plastic materials was recognized as a serious problem which could not be resolved without actual long term testing. Thus, the following test was initiated.

2.1 Storage of Gram Stain Reagents in Plastic Syringes

Sixteen commercially available sterile plastic disposable syringes made of polypropylene with a rubber tipped plunger were labeled with numbers 1 to 16. Each was weighed to the nearest milligram. The syringes were divided into four groups. Each group was filled with one of the following Gram Stain reagents and sealed with a plastic cap.

Gram Stain Reagents:

A Crystal violet

B Grams Iodine

C Denatured alcohol

D Aqueous Safranin

Each group was divided into duplicate sets, one set to be stored at room temperature and the other to be stored at 37°C. All syringes were weighed after filling, and one set every ten days thereafter for a period of 40 days. All syringes were examined periodically during the 40-day exposure. The alcohol containing syringes lost weight more rapidly than the others. Each week one of the syringe sets from each temperature group was used to stain both gram-positive and gram-negative micro-organism slide preparations.

Summary of test results:

1. Syringes containing Gram's Iodine began to turn yellow-brown after approximately ten days, and the iodine solution became colorless in less than 3 weeks.
2. After two weeks, Gram's iodine solutions stored at 37°C in the plastic syringes no longer yielded acceptable gram positive staining reactions.
3. The remainder of the Gram Stain reagents stored in the plastic syringes for 40 days at both room temperature and 37°C yielded acceptable gram stains.
4. Fluid loss even at 37°C, the worst case, was less than 0.5% in 40 days which could be considered negligible.
5. Syringes containing the crystal violet reagent and Safranin reagent began to show signs of freezing of the plunger after 40 days.

2.2 Storage of Gram Stain Reagents in All Glass Syringes

Two sets of Gram stain reagents were placed in a series of all glass syringes, capped with a plastic cap and stored at 37°C over a weekend. One set of

syringes was lubricated with a silicone grease while the other was not. Those syringes not containing the silicone grease were frozen with the exception of the alcohol syringes which had showed a significant loss in weight. Although the silicone coated syringes did not freeze over the weekend, leakage between barrel and plunger was observed for all syringes. In addition, moderate pressure on the plunger when the tip was blocked revealed a surprisingly amount of leakage between the glass barrel and the glass plunger.

2.3 Preliminary Test Conclusions

Plastic syringes are not suitable for long term storage of Gram's Iodine reagent. Plastic syringes have a slight tendency to freeze when Gram Stain reagents are stored in them for prolonged periods.

All glass syringes are unsuitable for storing Gram Stain reagents because of the freezing problems and the excessive leakage.

3.0 EVALUATION OF GLASS-TEFLON GAS TIGHT SYRINGES

Commercially available gas tight syringes having a glass barrel and a Teflon tipped plunger can be obtained with capacities up to 30 ml. Such syringes theoretically appeared to be the answer of the storage/dispenser concept for the slide stainer. A number of these syringes were purchased and evaluated with Gram stain reagents.

Two sets of the 30 ml glass-Teflon syringes were filled with the four Gram Stain reagents and the tip sealed with Teflon surfaced septum. One set was stored at room temperature while the other was stored at 37°C. The reagents used for the test were made 4 days previously to the start of the test and filtered through fine filter paper with the aid of vacuum. The Gram's Iodine was filtered through a Teflon filter.

Two analytical procedures for the iodine content of the Gram's iodine reagent were investigated. One consisted of diluting the Gram's iodine solution until an absorption reading could be obtained in a spectrophotometer at 350 mμ. This procedure did not yield meaningful data when a series of dilutions were prepared and absorption data obtained and plotted.

A second procedure, which has been successful, consisted in diluting the Gram's Iodine sample 1-20 with water, then extracting the iodine with carbon tetrachloride solution at 515 mμ. Calibrating solutions containing 0.5, 1.0, 2.0, 4.0 and 8.0 mg iodine per ml of carbon tetrachloride yielded a calibration curve which followed Beer's law. This analytical procedure was used to measure the iodine content of samples taken from each of the Gram's iodine solutions in the syringes and also of the stock solution which was stored in a tightly capped brown bottle. Analysis of the Gram's iodine solutions was carried out every 7 days for 49 days. Periodically all syringes were examined for leaks, and occasionally the Gram staining characteristics of the reagents in the syringes and those stored in a normal laboratory manner were tested by staining micro-organism slide preparations taken from fresh known cultures.

3.1 Results of Evaluation of Glass-Teflon Gas Tight Syringes with Gram Stain Reagents

The evaluation period lasted for approximately 73 days. The Gram stain reagents with the exception of the Gram's iodine did not appear to change when stored in the described syringes for the test period. The iodine content of the Gram's iodine reagent showed a decreasing level during the first 35 days for all methods of storage, but appears to have leveled off. This is shown in Figure 1. After 73 days, all Gram stain reagents stored in the glass-Teflon syringes yielded acceptable Gram stains of known micro-organisms.

The stability of the Gram's Iodine reagent stored in the glass-Teflon syringes can be predicted to be satisfactory for up to 280 days based on the limited data obtained. The stability of the other three Gram stain reagents stored in the described syringes should be stable for at least one year.

The Camco Quick Stain (Wright's Stain) has been stored in a plastic container for two years without affecting its performance. Therefore, its stability in the syringes can be expected to be similar.

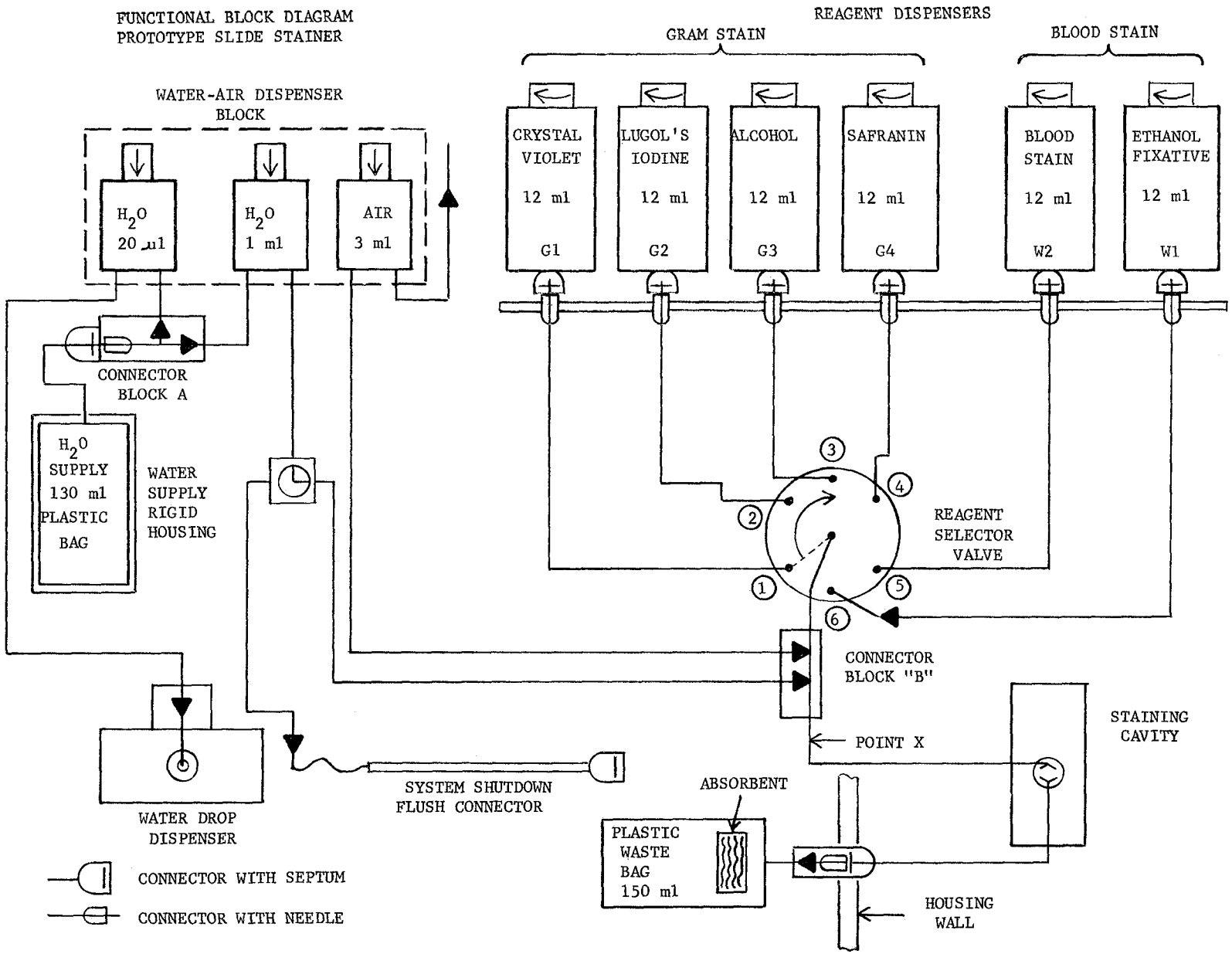


Figure 1. Functional Block Diagram Prototype Slide Stainer

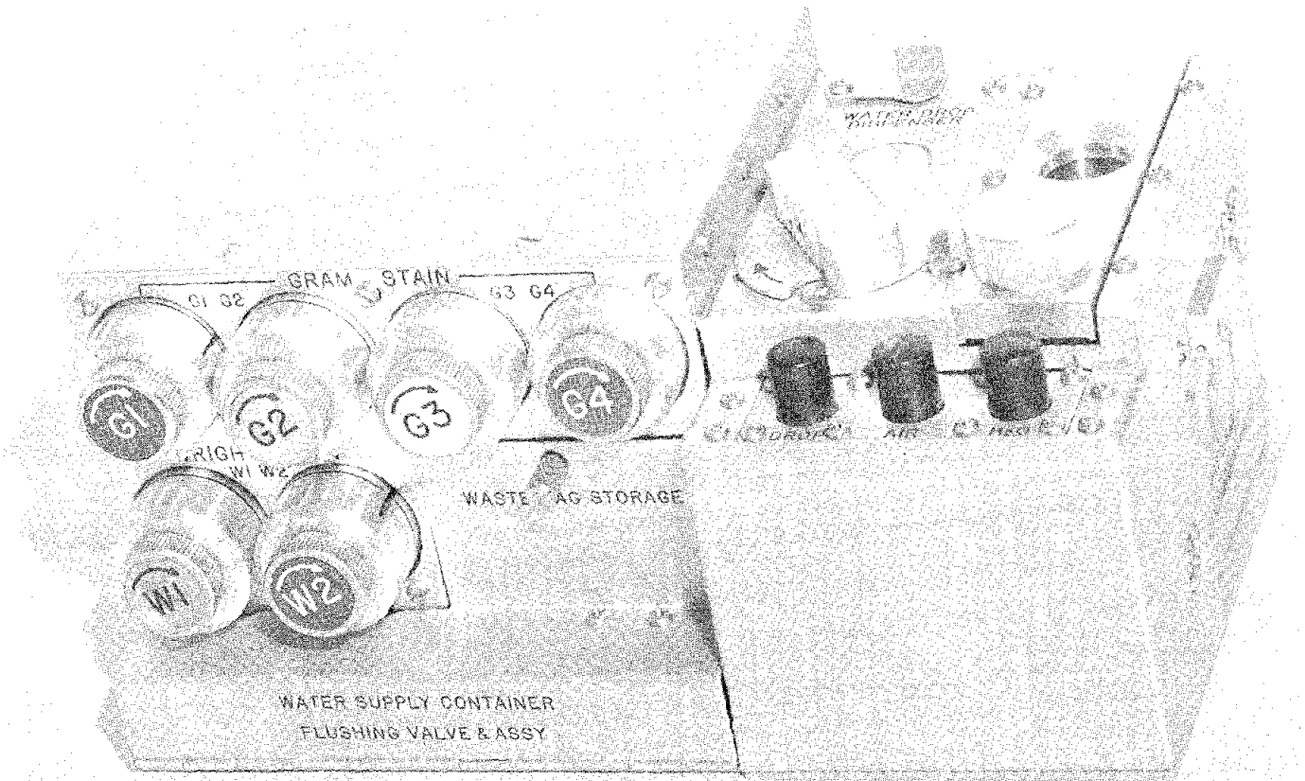


Figure 2. Prototype Slide Stainer

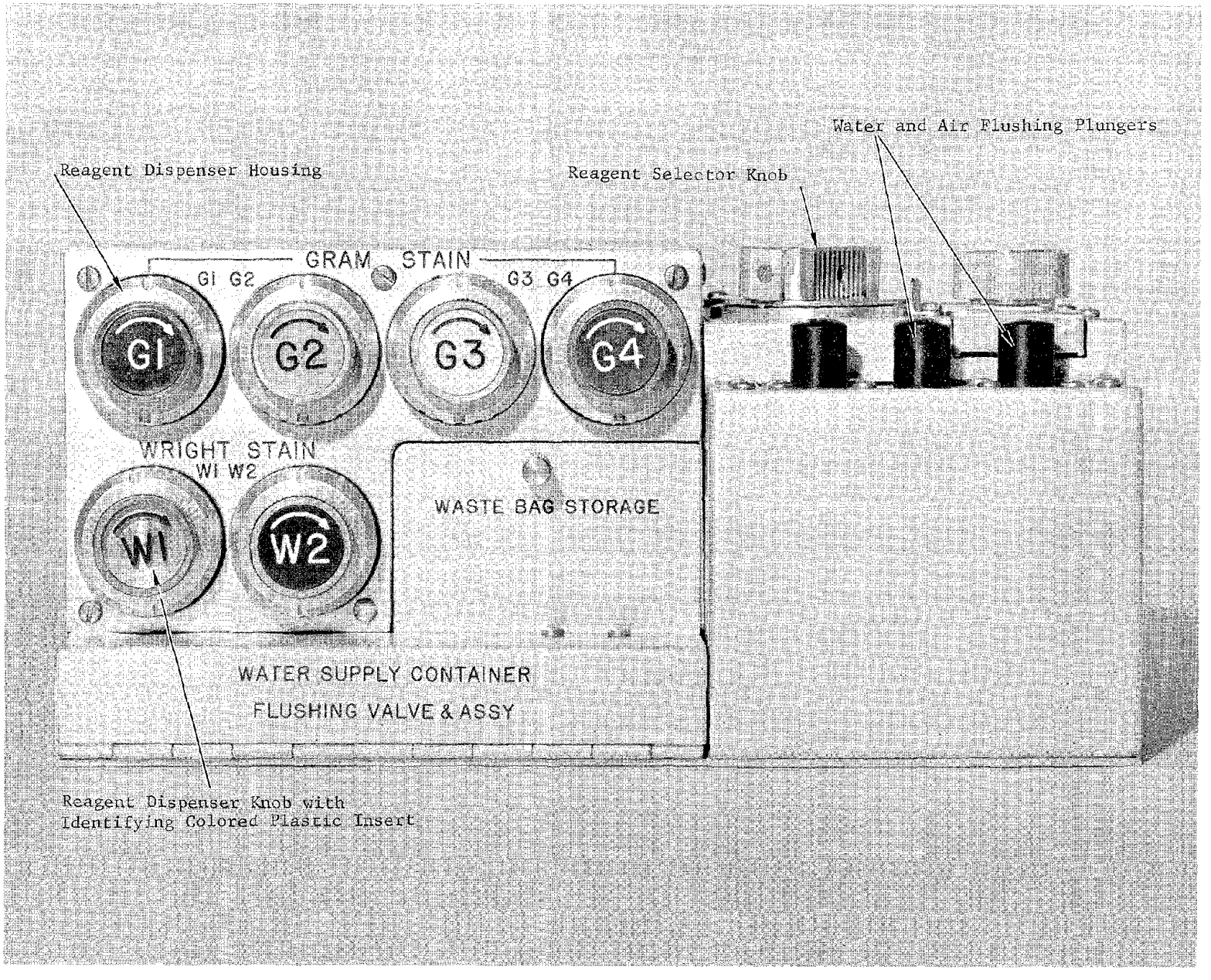


Figure 3. Front View of Prototype Slide Stainer

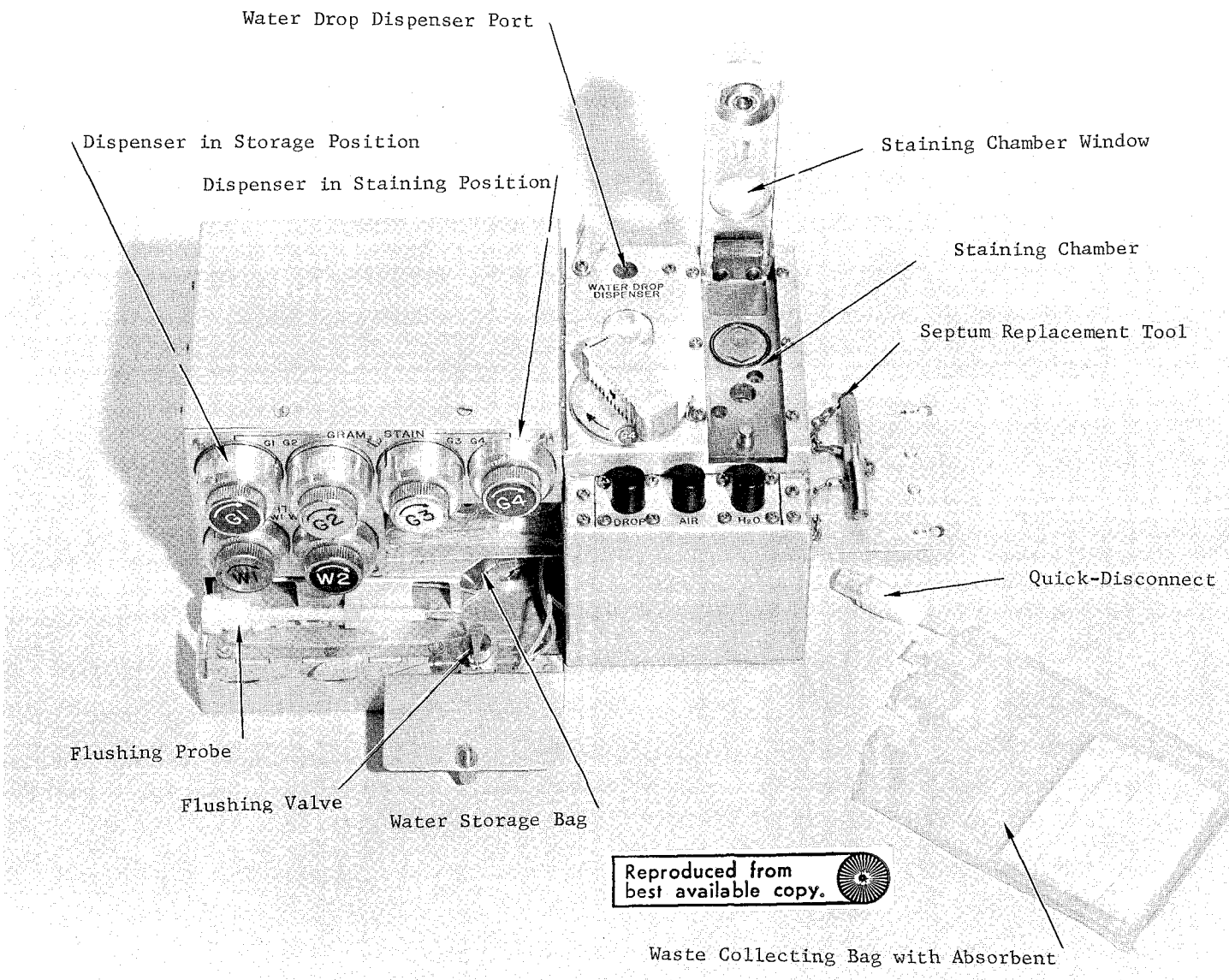


Figure 4. Slide Stainer with Access Doors Opened

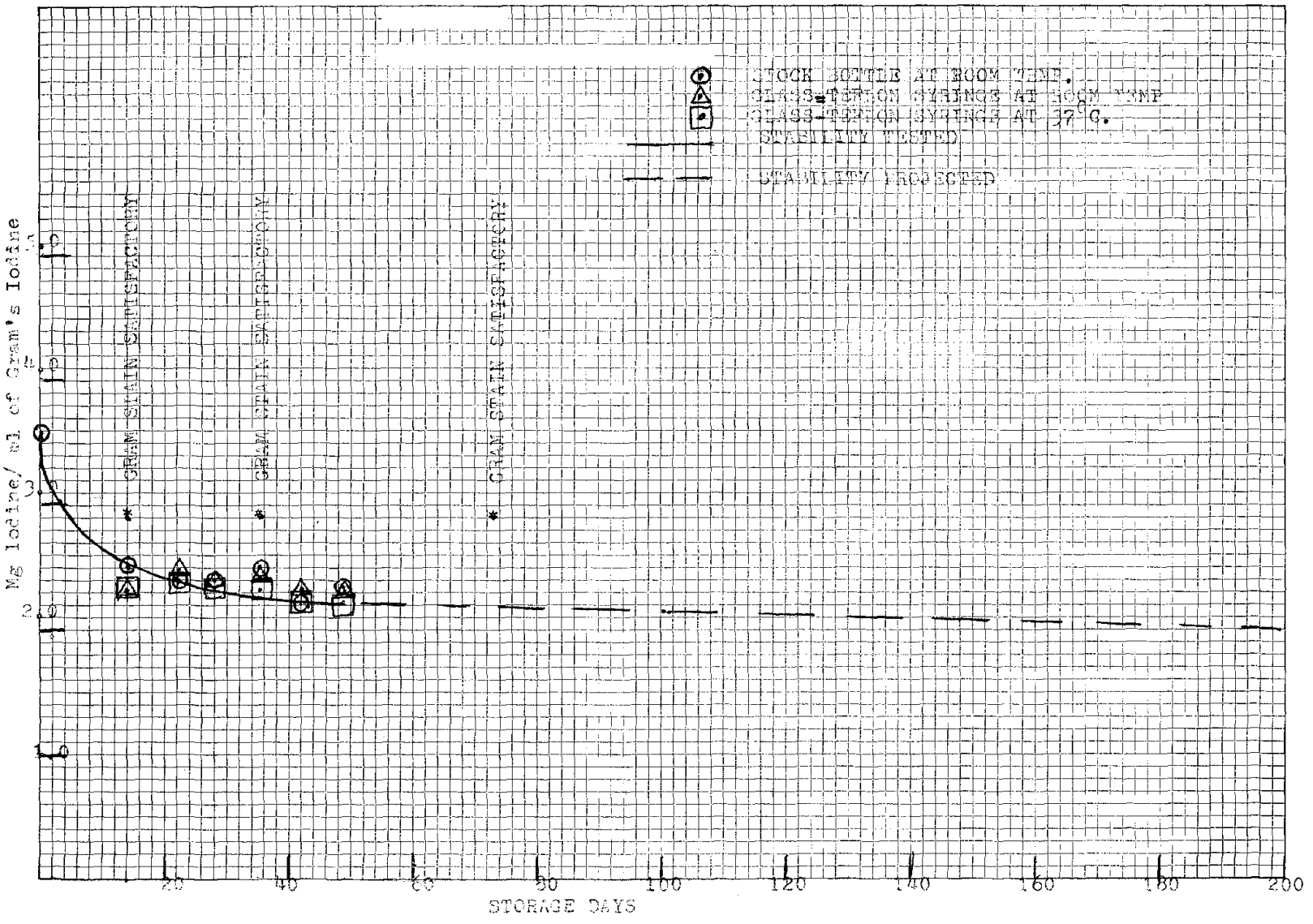
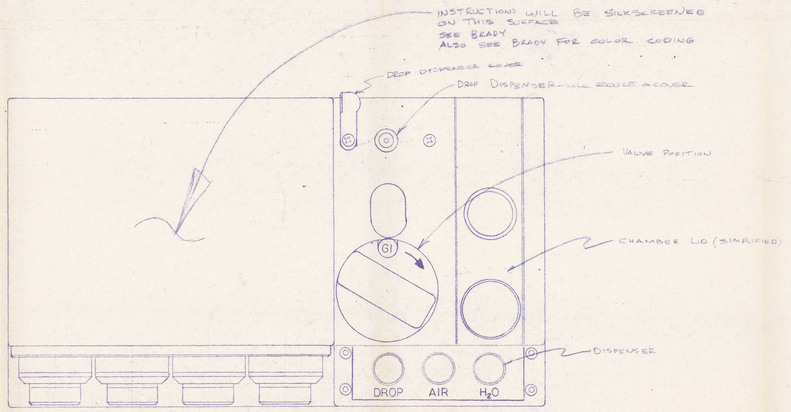
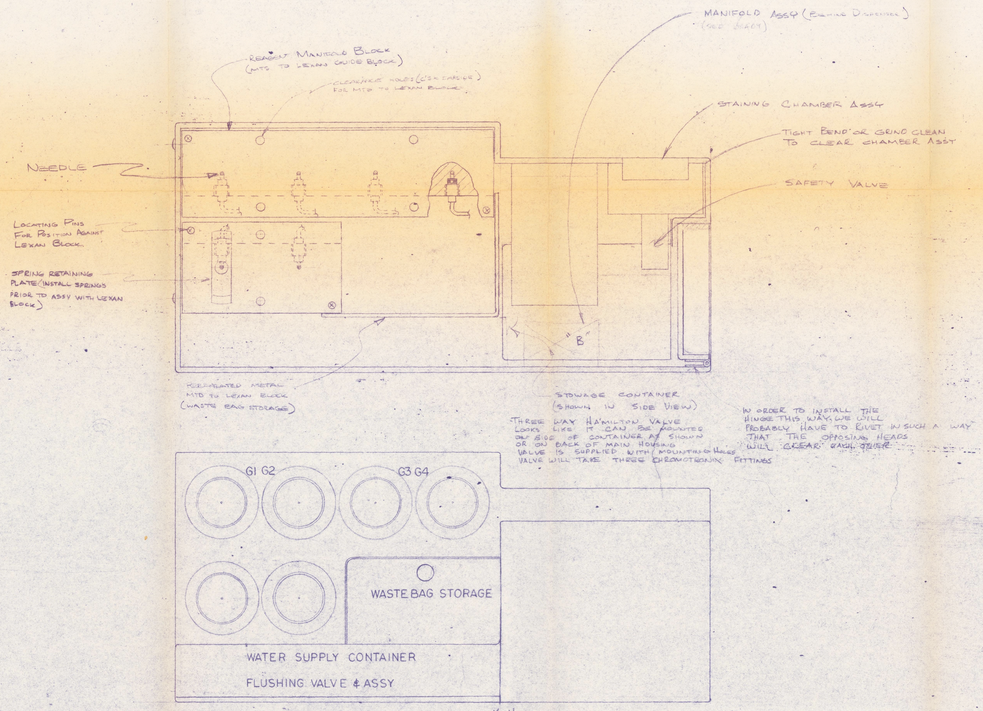
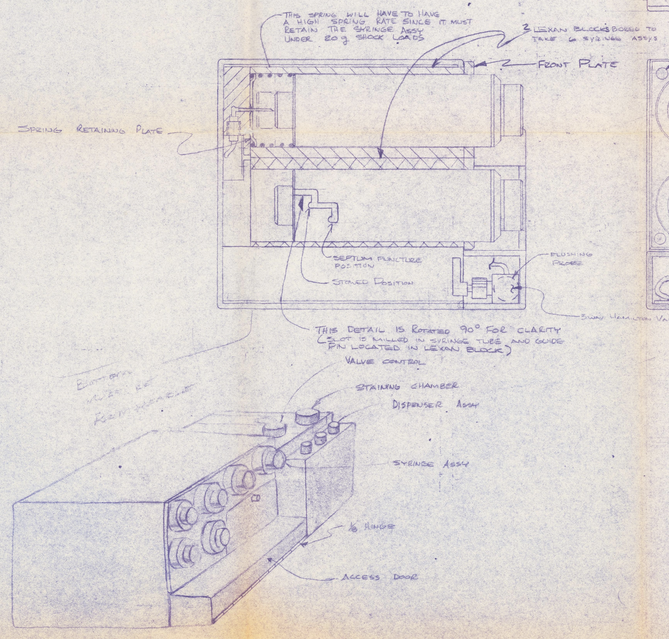


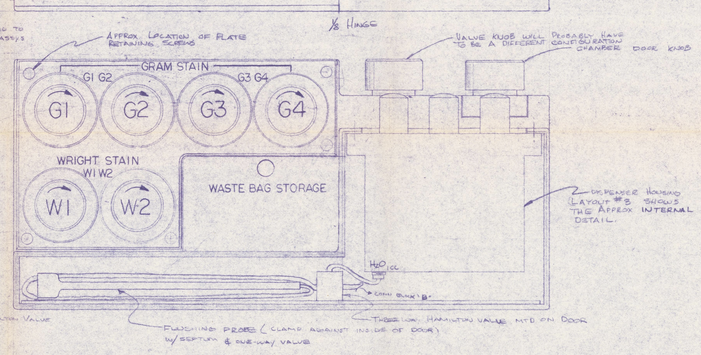
Figure E-1. Stability of Gram's Iodine

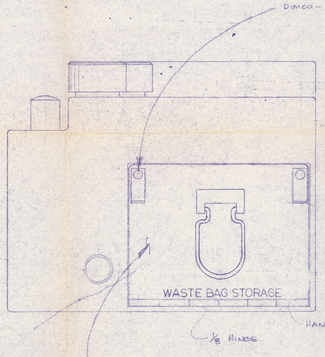
REVISIONS				
ZONE	LTR	DESCRIPTION	DATE	APPROVED

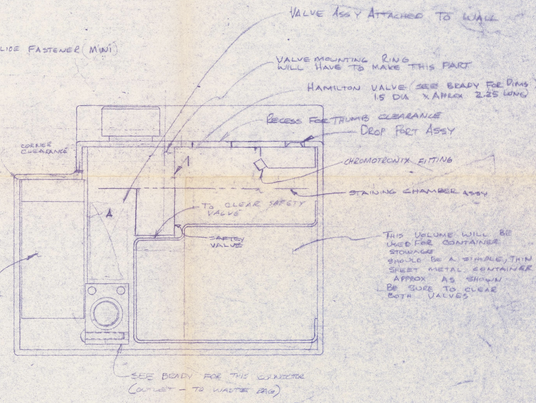












MAX DIME
9.2 × 5.3 × 4.3

LAYOUT # 1

QTY REQD	BECKMAN NO.	ITEM NO.	NOMENCLATURE OR DESCRIPTION	PART OR IDENTIFYING NO.	CODE IDENT.	MATERIAL	SPEC, NOTES, SIZES, VENDORS
			LIST OF MATERIALS OR PARTS LIST				

NEXT ASSY	USED ON	UNLESS OTHERWISE SPECIFIED	BY / DATE
APPLICATION		DIMENSIONS ARE IN INCHES TOLERANCES .X ± .050 ANGULAR ± 0° 30' .XX ± .020 .XXX ± .005 MACH. SURF. ✓ THREADS: CLASS 2A OR 2B REMOVE BURRS & SHARP EDGES .020 MAX. MACH. SURF. FLAT WITHIN .001 IN/IN OTHER SURF. FLAT WITHIN .005 IN/IN DO NOT SCALE DRAWING	DR
MATERIAL			CHK
			ENGR
			APPD
			APPD
FINISH			

Beckman® INSTRUMENTS, INC. A.T.O. 2500 HARBOR BLVD. FULLERTON, CALIF.

TITLE

SIZE	CODE IDENT NO.
E	05721

SCALE | SHEET

27-70-70-1