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NCHS NATIONAL CENTER FOR HEALTH STATISTICS

INSTRUCTION MANUAL

Data Collection

PART 16

HANES I

Hematology and Clinical Chemistry Procedures Developed or Utilized by the Center for Disease Control, Bureau of Laboratories, 1971-1975

U.S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
Public Health Service
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PREFACE

The First Health and Nutrition Examination Survey (HANES I) conducted by the National Center for Health Statistics between 1971 and 1975 had an extensive laboratory component. This manual covers all specimen collection activities and hematological and clinical chemistry procedures developed or utilized by the Center for Disease Control, Bureau of Laboratories associated with HANES I. Descriptions of serological testing procedures also performed by the Bureau of Laboratories and the T_3 and T_4 methodologies performed by National Health Laboratories, Inc., Seattle, Washington will be described in publications of findings covering these topics.

Appreciation for assistance in the preparation of this manual is gratefully given to the following areas of the Bureau of Laboratories, Center for Disease Control, Atlanta, Georgia: Nutritional Biochemistry Section, Clinical Chemistry Division; the General Hematology Section, Hematology Division; and Statistical Activities, Office of the Director.

CONTENTS

I. HANES I SPECIMEN COLLECTION

A. Blood

Collect the following Vacutainer tubes:

- *One 3-ml lavender-top EDTA tube* for hematological tests.
- *Three 15-ml red-top tubes and one 10-ml green-top (heparin) tube* for biochemical tests. (Collect a fourth 15-ml red-top tube from examinees designated for the Extended Study Panel.)

1. Venipuncture Technique[1]

   Perform venipunctures on all examinees. (If two puncture attempts are unsuccessful, perform a finger stick.) Clean the puncture site with an alcohol swab and allow site to dry. Apply the tourniquet and push the needle into the vein at a 15° angle. Loosen the tourniquet as soon as blood begins to flow from the needle. Fill a 3-ml lavender-top EDTA tube and three or four 15-ml red-top tubes. Withdraw the needle from the vein and press gauze firmly on the puncture site. Invert EDTA tubes to ensure proper mixing and allow blood in the red-top tubes to clot. If the subject has any adverse reaction during venipuncture, report that reaction to the physician.

2. Finger Stick Technique

   Perform finger stick, if necessary, by making a firm jab along the lateral aspect of the fingertip with a lancet. Wipe off the first drop of blood and collect blood for two differential slides, two white blood cell-hemoglobin (WBC-Hb) dilutions, and two hematocrit (Hct) tubes from succeeding drops. Wipe excess blood from the finger with a gauze after each specimen is collected. After collecting all specimens, press gauze over the puncture until bleeding stops. Immediately label and properly identify all specimens with examinee ID number.

B. Urine

Have examinees collect a casual urine specimen in a graduated 200-ml cup, using a U-bag for young children if necessary.

NOTE: A list of terms appears in the glossary.

II. SPECIMEN PROCESSING AND FIELD LABORATORY PROCEDURES

A. Hematology Specimens (reference 2 and pp. 161-166[3])

Use the well-mixed, lavender-top EDTA tube specimen for the hematology profile. Perform all tests in duplicate as soon as possible after the specimen is collected. If prompt processing is impossible, EDTA blood can be stored in the refrigerator up to 24 hours for red blood cell (RBC), WBC, Hb, and Hct determinations. Peripheral smears must be made within 1 hour after the specimen is collected.

1. Peripheral Smears (reference 2 and pp. 161-166[3])

   Make peripheral smears by placing a small drop of EDTA blood near the end of a slide. Allow a second pusher slide held at 30-40° angle to touch the drop of blood and push it smoothly toward the opposite end of the specimen slide. Allow the resulting thin film of blood to dry. Fix peripheral smears for 5 minutes in methyl alcohol and allow to dry again. Stain smears on the Ames Hema-Tek automatic slide stainer with a modified Wright's stain.[4]

2. Hematocrit (pp. 146-148[3])

   Determine hematocrit values by filling a capillary tube three-fourths full with blood, sealing it with clay sealant, and centrifuging it for a calibrated length of time for optimal packing of the red cells. Express the volume of erythrocytes as a fraction of the whole blood in a specimen as read with a microhematocrit reader. Run commercial controls daily.

3. Hemoglobin[5-7]

   Determine hemoglobin concentrations by making a 1:500 blood-Isoton dilution. Add a stromatolysing agent and hemoglobin reagent, and obtain a reading directly from the Coulter Hemoglobinometer.[5] Run commercial standards and controls daily.

4. Cell Counts (pp. 157, 262, 272, 275, 315-316[3] and references 8 and 9)

   Determine cell counts on the Coulter model Fn.[8] Obtain background counts of less than 100, and monitor the instrument daily with commercial controls. Dilutions should be made with either the Unopette system or the Coulter Dilutor II.[9] For white blood cell counts, make a 1:500 dilution of blood in Isoton and add a stromatolysing agent to lyse the red blood cells. Perform the instrument count and correct

the instrument value for coincidence if the count is greater than 10,000. For red blood cell counts, make a 1:50,000 dilution and correct the instrument count for coincidence. Calculate red blood cell indices and mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), and mean corpuscular hemoglobin (MCH) with the Best Anemia Classifier. Perform all tests in duplicate and record results on daily worksheets. Report abnormal cell counts to the physician.

B. Serum Specimens

1. Specimen Separation

a. Centrifugation

Allow blood in each red-top tube to clot for 30-45 minutes at room temperature. Remove the stopper, loosen clot from sides of tube with a clean wooden applicator stick, and replace stopper. Place tubes in centrifuge carriers, balance, and centrifuge at 2,400 rev/min for 10 minutes.

b. Pooling

Do not allow serum to remain in contact with clot longer than 1 hour after the specimen is collected. Remove serum from all centrifuge tubes with a Pasteur pipette and pool it into a clean, 20-ml red-top tube, carefully avoiding the introduction of any cellular debris. Stopper tube, and mix contents by inverting tube. Pooled serum can be refrigerated up to 4 hours if necessary before specimens are allocated.

c. Guideline for adequate specimens

If the serum from any red-top tube becomes grossly hemolyzed (e.g., from traumatic venipuncture, mishandling), do not pool it with serum from remaining tubes. Using clear serum only, fill as many vials as possible. If all the serum from an examinee is turbid, lipemic, or icteric, pool and allocate as usual (not all biochemical tests can be performed on these specimens).

2. Specimen Allocation

a. Health and Nutrition Examination Survey I (HANES I) (stands 1-65)

Label all vials with examinee ID number and allocate each examinee pooled serum into 3-ml Microdel vials as follows:

- Vial 1 (serum folate)
  1 ml serum + 1 mg ascorbic acid added as a preservative
- Vial 2 (plasma vitamin C)
  0.5 ml serum + 2.0 ml 6% metaphosphoric acid
- Vial 3 (serum vitamin A, total protein, albumin)
  3 ml serum
- Vial 4 (serum iron, total iron-binding capacity, magnesium, cholesterol)
  3 ml serum
- Vial 5 (reserve vial for repeat analyses)
  3 ml serum

- Vial 6 (Extended Study Panel—biochemical constituents)
  3 ml serum
- Vial 7 (Extended Study Panel—serological constituents)
  3 ml serum
- Vial 8 (see section C.2.)
- Vial 9 (Extended Study Panel—serum T_3 and T_4)
  4 ml serum (Scientia 5-ml tube with rubber stopper)

b. Health Examination Survey (HES) (stands 66-100)

Label all vials with examinee ID number and allocate each examinee pooled serum into 3-ml Microdel vials as follows:

- Vial 1 (serum folate)
  1 ml serum + 1 mg ascorbic acid added as a preservative
- Vial 2 (plasma vitamin C)
  0.5 ml serum + 2.0 ml 6% metaphosphoric acid
- Vial 3 (Extended Study Panel—biochemical constituents)
  3 ml serum
- Vial 4 (serum sodium, potassium, magnesium)
  3 ml serum
- Vial 5 (serum cholesterol)
  3 ml serum
- Vial 6 (Extended Study Panel—serological constituents)
  3 ml serum
- Vial 7 (reserve vial for repeat analyses)
  3 ml serum
- Vial 8 (see section C.2.)
- Vial 9 (Extended Study Panel—serum T_3 and T_4)
  4 ml serum (Scientia 5-ml tube with rubber stopper)

After serum is allocated, insert tips into vials and screw on caps tightly. Freeze vials promptly (−20° C).

C. Urine Specimens

1. Screening Procedure

Screen urine specimens for albumin, glucose, and pH, using a Combistix. Record results on daily worksheets.

2. Analytical Preparation (HANES I, Stands 1-65)

- Vial 8 (urine riboflavin, thiamine, iodine, creatinine)
  Label a 50-ml Wheaton vial with examinee ID number. Fill vial approximately two-thirds full with urine, if enough is available. Add 1 drop of glacial acetic acid and mix. Using pH test paper, check for urine pH below 4.5. If necessary, add more acetic acid drop by drop, rechecking until a pH less than or equal to 4.5 is reached. Place the stopper on the vial, pull skirt of stopper tightly over the neck of the vial and freeze promptly (−20° C).

III. SPECIMEN SHIPPING INSTRUCTIONS

A. Biochemical Specimens

Place all vials of serum and urine specimens from an examinee into a plastic bag, and keep frozen until they are shipped. Make shipments to the Center for Disease Control (CDC) daily, if possible. Collect examinee specimen bags for each day, pack securely in an insulated styrofoam shipper placed atop a cardboard box containing 12 lb of solid-slab dry ice, and insert the styrofoam lid. Place related examinee data cards in a plastic envelope on top of the foam lid, place outer carton lid on top of shipper and fasten it securely with straps or filament tape. Attach franked label and ship by air express.

B. Hematological Specimens and Smears

Send the remaining whole blood specimens in the 3-ml lavender-top tubes to CDC for hemoglobinopathy studies. Pack them securely in a small insulated shipper with a coolant to keep the specimens chilled but not frozen. Close lid, attach franked label, and ship daily to CDC. At the end of each stand, place the labeled, stained smears in a slide box and ship to CDC for microscopic examination.

C. T_3 and T_4 Specimens

Place 5-ml tubes of frozen serum into 10-slot styrofoam holders and mail to Scientia Laboratories in preaddressed envelopes. Ship each styrofoam holder separately with accompanying report papers every 3 weeks.

IV. HEMATOLOGICAL METHODOLOGIES

A. General

This section contains a summary of the hematological and quality control procedures performed in the field operations and in the CDC Hematology Division in support of HANES I and HES. The laboratory procedures are condensed into a short statement of the analytical principle, a brief description of the procedure, criteria for repeating the procedure in the field and for calling results to the attention of the examining physician, and listing the material used for quality monitoring of the procedure. Citations of published reports are included.

Quality monitoring data are sent by field personnel to the CDC Hematology Division and reviewed.

B. Hematological Laboratory Procedures

1. Microhematocrit

The hematocrit reading measures the volume of erythrocytes in a given volume of whole blood. A specimen of blood is centrifuged at a speed and for a length of time calibrated for optimal packing of the red blood cells. After centrifugation, the volume of red cells relative to the volume of whole blood in the specimen is read.

Procedure.—Capillary tubes are filled to not less than 1 or 2 cm from the end by gravity and capillarity. The empty end of the capillary tube is sealed with clay sealant. The filled tubes are balanced in numbered radial grooves in the microhematocrit centrifuge head, the sealed end in contact with the peripheral rim. The cover is replaced and the capillary tubes are spun at 10,000 rev/min for 5 minutes.

The hematocrit value is read with a microhematocrit reader. Each specimen is run in duplicate, and the results must agree within 0.02 (2%). Samples with mean values below 0.30 (30%) and above 0.55 (55%) are retested, and the results are called to the attention of the physician.

The centrifuge is monitored daily with commercially available control materials. Maintenance is performed at each stand.

2. Hemoglobin

Hemoglobin concentrations are determined on the Coulter Hemoglobinometer.[5] The procedure is based on the hemiglobin-cyanide (cyanmethemoglobin, HiCN) principle. A flow-through cuvette is used. A beam of light passes through the fluid into a

photoelectric measuring device. The amount of light transmitted by a reference solution is measured and stored. The amount of light transmitted by the lysed, diluted, and converted blood sample is compared with that transmitted by the blank, and the hemoglobin concentration is automatically computed and displayed as a numerical readout in grams per deciliter (g/dl).

Procedure.—First, 40 μl of blood is aspirated by a Coulter Dilutor II and dispensed into 20 ml of Isoton, resulting in a 1:500 dilution. A stromatolyzing and hemoglobin-conversion reagent (Zap-Oglobin, 6 drops) is added and a white cell count performed. The remaining solution is poured into the cuvette, and the numerical reading in g/dl obtained. Duplicate dilutions are tested, and results must agree within 0.2 g/dl. Samples with mean values below 11.0 and above 18.5 g/dl are retested and results are called to the attention of the physician.

The hemoglobinometer is checked daily with commercially available hemoglobin reference solutions.

3. Cell Counts

Red and white blood cell counts are determined on the Coulter Fn, operated according to the *Instruction and Service Manual for Coulter Fn.*[8] A specific concentration of particles suspended in an electrolyte solution is forced by a mercury siphon through an aperture of specific dimensions. A current flows between an electrode inside the aperture tube and a second electrode outside the tube. As a particle passes through the aperture, an equal volume of electrolyte is displaced and the resistance in the path of the current changes. This produces a voltage drop, the magnitude of which is proportional to the volume of the particle. The voltage pulses are fed into a threshold circuit, which differentiates them by generating count pulses for only the particles that exceed the threshold level, thus counting the number of particles in passage. A correction factor for coincidence must be employed for counts over 10,000.

Procedure.—In order to obtain the white cell count, 40 μl of blood is aspirated by a Coulter Dilutor II and dispensed into 20 ml of Isoton for a 1:500 dilution (see Instruction Manual[9]). From this solution, 202 μl is aspirated by the Coulter Dilutor II and dispensed into 20 ml of Isoton, resulting in a final dilution of 1:50,000 for the red cell count.

(a) *The instrument count for red cells* is made on the 1:50,000 dilution. This value (corrected for coincidence counting with a Coulter Coincidence Chart) is multiplied by the appropriate factor to obtain the red cell count $N \times 10^{12}/l$($N \times 10^{6}$/ μl). Duplicate dilutions are tested, and results must agree within $0.2 \times 10^{12}/l$. Mean values below 3.5 and above $6.0 \times 10^{12}/l$ are repeated and called to the attention of the physician.

(b) Six drops of Zap-Oglobin are added to the 1:500 dilution, and *the instrument count for white cells* is performed. Corrections are made for readouts greater than 10,000 with the Coulter Coincidence Chart. The (corrected) readout is multiplied by the appropriate factor to obtain the white blood cell count in $N \times 10^{9}/1$ ($N \times 10^{3}/\mu l$). Duplicate dilutions are tested, and results must agree

within 0.4 X 10^9/l. Samples with mean values below 3.0 and above 15.0 X 10^9/l are retested and results are called to the attention of the physician.

The instrument is monitored daily with commercially available control materials. Background counts are less than 100, and maintenance is performed at each stand.

4. Differential White Blood Cell Counts and Red Blood Cell Morphology

Differential counts are performed on the manually prepared and stained (Ames Hema-Tek Automatic Slide Stainer) wedge smears from HANES I examinees participating in the detailed examinations (stands 1-65) and smears taken from all HES examinees (stands 66-100). One hundred white blood cells are classified using a microscope with a 100X oil-immersion lens. Two hundred white blood cells are classified on those specimens with abnormal differentials. Red blood cell morphology is graded as normal or abnormal on a scale of 1+ to 4+, and other miscellaneous findings are recorded. Platelets are described as normal, increased, or decreased.

C. Hemoglobinopathy Screening Protocol

The EDTA tubes (from stands 37-100) are sent to the CDC Hematology Division to be screened for hemoglobinopathies. Cellulose acetate electrophoresis is performed on all specimens. Those specimens that appear abnormal electrophoretically are then tested as outlined in the flowchart (figure 1). Hemoglobin A_2 quantitation is performed on all specimens that have MCV values less than 80 fl (μ^3). This quantitation result is correlated with iron levels to diagnose β-thalassemia presumptively.

1. Cellulose Acetate Electrophoresis[13,14]

Electrophoresis is the movement of charged particles in an electrical field. At pH 8.4-8.6, hemoglobin is a negatively charged protein; the hemoglobin, therefore, migrates toward the anode in an electrical field with cellulose acetate as the support medium. During electrophoresis, various hemoglobins separate as a result of charge differences caused by structural variation. This separation allows identification of different hemoglobins. Specimens for hemoglobinopathy screening include those specimens from stands 37-100.

Procedure.—All specimens are prepared for cellulose acetate electrophoresis screening by washing the red cells with saline and then lysing them with EDTA/water hemolysate reagent. Tris-EDTA-borate buffer (TEB) at pH 8.4 is used in the chambers and to soak the wicks and plates. Hemolysate specimens are applied to the cellulose acetate plates and electrophoresed at 450 V for 20 minutes at room temperature. Plates are stained with 0.5% Ponceau S, washed in 5% acetic acid, fixed in absolute methanol, and cleared in 20% glacial acetic acid in absolute methanol.

A control hemolysate containing four known hemoglobins is applied to each strip or plate with the unknown specimens. The patterns of the unknown specimens are compared with those of the known hemolysates. Specimens are standardized by adjusting hemoglobin concentrations to relatively equal levels and by applying specimens with a uniform applicator. All specimens that appear normal electrophoretically and specimens with an MCV less than 80 fl (μ^3) are tested further.

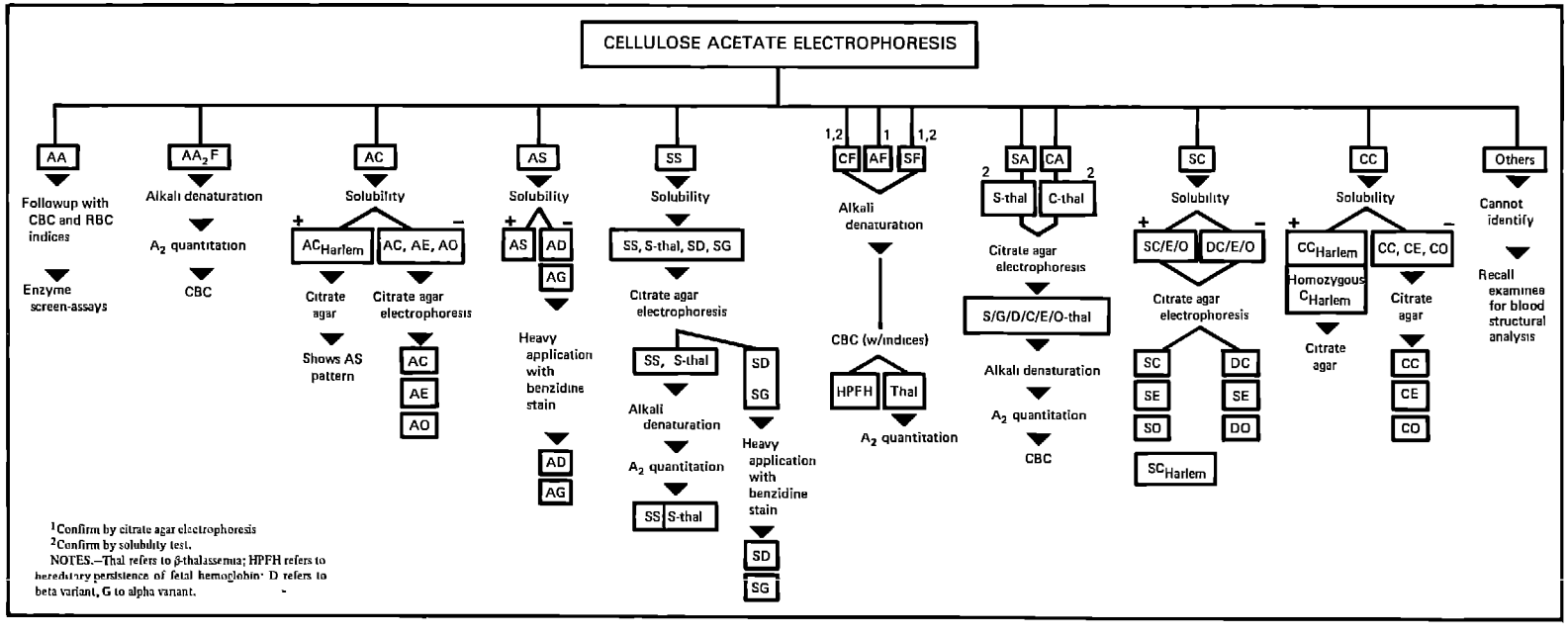

Figure 1. Flowsheet for laboratory diagnosis of hemoglobinopathies

2. Alkaline Globin Electrophoresis[14,15]

At pH 8.6-8.7, the globin chains of hemoglobin migrate to different areas on a support strip of cellulose acetate placed in an electrical field. Prior to this electrophoretic reaction, 2-mercaptoethanol is added to separate the heme groups from the globin chains of the hemoglogin and urea is added to separate the α and non-α chains.

Procedure.—Stock veronal buffer, pH 8.6, is diluted 1:1 with deionized, distilled water, to prepare a working buffer. 180 g of urea is added to 300 ml of the working veronal buffer. 50 μl of the working buffer, 40 μl of 2-mercaptoethanol, and 50 μl of specimen are mixed, covered, and refrigerated for 2 to 2½ hours. The remaining buffer containing 2.5 ml of 2-mercaptoethanol is used to soak the cellulose acetate strips and fill the chamber. Electrophoresis is performed for 80 minutes at 2 mA per strip (300 V) with the chambers on ice packs. Strips are then stained with 0.5% Ponceau S for 3 minutes and rinsed with 5% acetic acid.

A control hemolysate containing two known hemoglobins is applied to each strip or plate with the unknown specimens. The patterns of the unknown specimens are compared with those of known hemolysates. Specimens are standardized by adjusting hemoglobin concentrations to relatively equal levels (3-6 g/dl) and by applying specimens with a uniform applicator.

3. Acid Globin Electrophoresis[14,15]

The heme groups are separated from the globin chains of hemoglobin with 2-mercaptoethanol, and the α and non-α chains are separated with urea. The heme groups migrate off the strip and the globin chains separate during the amount of time allotted for this electrophoresis. Because globin chains differ in their electrical charge at pH 6.0-6.2, they migrate to different areas on a support strip of cellulose acetate in an electrical field.

Procedure.—72 g of urea is dissolved in 120 ml of stock buffer (10.2 g of Tris, 0.6 g of EDTA, 3.2 g of boric acid in 1 l, pH 8.4), and the pH is adjusted to 6.0 with 30% citric acid. The volume is adjusted to 200 ml with deionized water. 100 ml of this buffer is used to dilute the specimens and soak the plates. 10 μl of specimen, 10 μl of deionized water, 20 μl of buffer, and 10 μl of 2-mercaptoethanol is added to a small test tube and incubated at room temperature for 30 minutes. 0.5 ml of 2-mercaptoethanol is added to the remaining buffer and is used to fill the chambers and soak the cellulose acetate strips. Specimens are applied in the center of the strip and electrophoresed for 2 hours at 250 V at room temperature. Strips are stained with 0.5% Ponceau S for 3 minutes and rinsed with 3% acetic acid.

A control hemolysate containing two known hemoglobins is applied to each strip with the unknown specimens. The patterns of the unknown specimens are compared with those of known hemolysates. The type is standardized by adjusting hemoglobin concentrations to relatively equal levels (3-6 g/dl) and by applying specimens with a uniform applicator.

4. Citrate Agar Electrophoresis[16]

Citrate agar electrophoresis involves the movement of hemoglobins (charged proteins) in an electrical field with 1% agar as the support medium; the working buffer is 0.005 mol/l sodium citrate, pH 6.0-6.2. The separation of various hemoglobins on this medium is based on electrophoretic charge and on adsorption of the hemoglobin to the agar (or to its impurities). This adsorption may be related to hemoglobin solubility. Therefore, the system differs from that of a system with migration based mainly on electrophoretic charge.

Procedure.—Clean glass microscope slides are precoated with 0.1% agar melted in distilled water. Slides are covered with 1% agar (1 g agar in 100 ml citrate buffer). Carbon-tetrachloride-washed-cell hemolysates are prepared and diluted to a concentration of less than 1 g/dl. Stock 0.05 mol/l citrate buffer diluted 1:10 with deionized distilled water and adjusted to pH 6.0-6.2 with 30% citric acid is used to soak wicks and to fill the chamber compartments. Hemolysates are applied to plate centers. Plates are placed agar-side down on the wicks across the chamber, and 15-17 mA of current per plate (70-90 V) is applied for 70 minutes. Citrate agar electrophoresis is performed with the chambers on ice packs. Plates are stained with a tetramethyl-benzidine solution diluted 1:1 with distilled water and several drops of 30% hydrogen peroxide and are washed in acidified water (1 drop concentrated HCl in 500 ml of water).

A control hemolysate containing four known hemoglobins is applied to each plate with the unknown specimens. The patterns of the unknown specimen are compared with those of the known hemolysates.

5. Solubility Test[17,19]

Sickle hemoglobin (Hb S) in the reduced state will not dissolve but forms a precipitate in a high-phosphate buffer solution. In sickle cell anemia most of the hemoglobin sickles and is insoluble. Hemoglobin A interacts with hemoglobin S and precipitates with it, producing an AS heterozygote.

Procedure.—Solubility testing is performed on whole blood mixed with dithionite reagent. A test tube containing the specimen is held 2.5 cm from the front of a white card with black lines. The test is read as positive for sickling hemoglobin if the black lines on the white card are not visible through the solution. Hb C Harlem, Hb Travis, and possibly some other hemoglobins, as well as Hb S, give positive solubility test results. Conversely, the test is read as negative for sickling hemoglobin when the black lines are visible through the blood-reagent solution.

Known positive and negative controls are run with each group of unknowns being tested for solubility.

6. Alkali Denaturation Test for Fetal Hemoglobin[20]

Fetal hemoglobin resists denaturation by a strong alkali more than do other hemoglobins. A strong alkali added to a hemolysate containing a known amount of hemoglobin causes denaturation to occur. After a specified time, the denaturation is

stopped by adding half-saturated ammonium sulfate, which lowers the pH and precipitates the denatured hemoglobin. After the precipitate is filtered out, the amount of unaltered hemoglobin is measured and expressed as the percentage of alkali-resistant (fetal) hemoglobin.

Procedure.—Total hemoglobin is measured by reading the optical density (OD) of a solution of hemolysate in distilled water at 540 nm. Hemolysates are made from red cells washed with carbon tetrachloride. 1.6 ml of 1/12 mol/l KOH (pH 12.7) is incubated at 20° C for 10 minutes before 100 μl of the hemolysate is added and 3.4 ml half-saturated ammonium sulfate is added after 1 minute. This solution is filtered, and the OD of the filtrate is read at 540 nm. The test is run in duplicate, and the results are calculated with the following formula:

$$\% \text{ fetal hemoglobin} = \frac{\text{OD fetal Hb}}{\text{OD total Hb}} \times 0.203 \times 100.$$

Known normal and abnormal hemolysates are run with each set of specimens. The OD for each fetal Hb and total Hg set of duplicates must not differ by more than 0.03.

7. Fluorescent Spot Screening Test for Glucose-6-Phosphate Dehydrogenase (G-6-PD)[21,22]

Glucose-6-phosphate dehydrogenase catalyzes the oxidation of glucose-6-phosphate (G-6-P) in the glucose metabolism pathways and subsequently reduces $NADP^+$ to NADPH by transferring two electrons from G-6-P. The reduced NADPH fluoresces when exposed to longwave ultraviolet (UV) light. Thus, when whole blood is mixed with the G-6-PD screening reagent, the reduction reaction occurs when G-6-PD is present (i.e., in normal cells), and the resulting fluorescence can be viewed under a longwave UV light.

Procedure.—0.01 ml of whole blood is mixed with 0.1 ml of the Hyland G-6-PD Screening Reagent, and a control spot is immediately applied to filter paper. The blood-reagent mixture is then incubated at 37° C for 3 minutes, after which another spot is made. The dried spots are examined under longwave UV light.

Normal blood specimens fluoresce strongly. The control spot and specimens with G-6-PD deficiency either fluoresce weakly or not at all.

8. Microchromatography for Hemoglobin A_2 (Hb A_2) Quantitation[23,24]

In certain buffer systems, the interaction of charged groups on the ion-exchange resin and charged groups on the hemoglobin molecules causes the separation of different hemoglobin fractions seen in ion-exchange chromatography. The hemoglobin fractions are eluted from a column and quantitated.

Procedure.—Disposable Pasteur pipettes are plugged with cotton and packed to a height of 6 cm with DE 52 anion exchange resin. 1 drop of hemolysate is diluted with 6 drops of 0.05 mol/l Tris-HCl buffer, pH 8.5, and applied to the top of the column, after which 0.05 mol/l Tris-HCl buffer, pH 8.3, is continuously applied. The

effluent dripping from the bottom of the column is collected when the Hb A_2 begins to move away from the remaining hemoglobin. After the Hb A_2 is eluted, the buffer on top of the column is changed to 0.05 mol/l Tris-HCl,pH 7.0. The effluent containing the remaining hemoglobin is collected in a 25-ml volumetric flask. The absorbance of the eluted Hb A_2 and the effluent is read at 415 nm. The percentage of Hb A_2 is calculated with the following formula:

$$\frac{\text{OD Hb } A_2}{\text{OD Hb } A_2 + (2.5 \times \text{OD R})} \times 100 = \%\ \text{Hb } A_2$$

(R = remaining hemoglobin).

When slowly moving fractions such as Hb S are present, the Tris-HCl method is altered as follows:

(a) The column (a 5-ml disposable pipette can be used) is packed to 16 cm.
(b) Hb A_2 is eluted with pH 8.35 buffer.
(c) Hb S is eluted with pH 8.2 buffer.
(d) Tris-HCl buffers and DE 52 resin are used.

The percentage of the Hb A_2 is calculated with the following formula:

$$\frac{\text{OD Hb } A_2}{\text{OD Hb } A_2 + (2.5 \times \text{OD S}) + (2.5 \times \text{OD R})} \times 100 = \%\ \text{Hb } A_2$$

(S = slow-moving variant such as Hb S).

Because Hb A_2 cannot be separated from Hb C, Hb E, or Hb O, Hb A_2 cannot be quantitated when Hb C, Hb E, or Hb O are present.

Normal and elevated controls are run with each set of specimens.

9. Isopropanol Precipitation Test (p. 293[12] and references 25 and 26)

The three factors that primarily control the stability of hemoglobin are: bonding within the globin subunit, bonding of the globin to the heme, and bonding among the hemoglobin chains. Hemoglobin may become less stable when any of these bonds are altered. This stability can be demonstrated by exposing hemoglobin to heat, because normal hemoglobin in an appropriate buffer precipitates only when slightly heated to a specific temperature, whereas unstable hemoglobins treated in the same manner are denatured.

Procedure.—Hemolysates are prepared by washing the cells three times with isotonic saline by centrifuging at 3,000 rev/min for 3 minutes. Equal volumes of distilled water and packed cells and a half volume of carbon tetrachloride are mixed and centrifuged, and the hemolysate is removed. One volume of 2% KCN is added to five volumes of hemolysate. In a stoppered test tube, 2 ml of buffer (12.1 g of Tris and 170 ml of isopropyl alcohol in 1 l, adjusted to pH 7.4 with concentrated HCl) is allowed to equilibrate to 37° C. 0.2 ml of hemolysate is then added to the buffer and incubated at 37° C for 30 minutes. Precipitation in the tube is then measured after 5 minutes and 30 minutes. The normal specimen will still be clear after 5 minutes, whereas one containing an unstable hemoglobin will have begun to show a

precipitate. A flocculent precipitate will form within 30 minutes if an unstable hemoglobin is present; a normal specimen, however, will remain clear.

A known normal blood sample drawn at approximately the same time as the test specimen is run as a normal control. If a specimen with an unstable hemoglobin is available, it is run as an abnormal control; otherwise, a cord blood hemolysate without KCN added is run as an abnormal control.

V. BIOCHEMICAL METHODOLOGIES

A. General

The following section contains summaries of the laboratory procedures and quality control data for the biochemical tests performed in the Clinical Chemistry Division of the Center for Disease Control for HANES I and HES.

1. Format for Reporting Laboratory Procedures

Laboratory procedures are written as abstracts that contain: (a) a statement of the analytical principle applied in quantitating each analyte, (b) a brief description of each laboratory procedure including calibration and quality control scheme, and (c) citations of published reports and technical modifications made by CDC on laboratory methods used.

2. Quality Control Data

Two categories of data are available for estimating analytical error. The first concerns bench quality control samples and is used to estimate the inherent errors in instruments, reagents, calibration procedures, and laboratory procedures. Most importantly, these data provide estimates of the magnitude of any time-associated trends in an analytical method. They do not, however, provide best estimates of total measurement error of the HANES I analytical system because:

a. Bench quality control samples are not subject to sources of error inherent in the additional sample handling and processing steps in the system.

b. Bench quality control samples provide a means to standardize reagents, instrument calibration, instrument malfunction, and laboratory procedures. If an observed value on a bench quality control sample, or the average of several observed values, falls outside established control limits, both controls and samples of unknown concentration are reanalyzed. If the control limits have been properly established, this procedure automatically truncates the distribution of determinations, even when the measurement system is statistically controlled. Therefore, estimates of imprecision based on these data will underestimate the true imprecision in the process. If the system is not operating in a state of statistical control (i.e., if there are uncontrollable time-associated trends) or if the control material is either unstable or nonhomogeneous, it is difficult to interpret the common measures of imprecision (standard deviation or coefficient of variation).

The second category of data is that of quality surveillance data, obtained either by repeatedly submitting samples coded as regular HANES I samples but all taken from the same serum pool, or by resubmitting aliquots from HANES I examinee specimens that had already been analyzed. Because this blind surveillance system implemented in the spring of 1973 is not available for every analyte in HANES I, and because we cannot cite a publication describing the estimation technique used for data collected on sample resubmissions, quality surveillance data are not included in this summary report. (Our quality surveillance data did tend to confirm that the estimates of imprecision from bench quality control samples are reasonable and appropriate.)

3. General Aspects of Data Analysis and Presentation

For each laboratory method and each quality control pool, a one-way classification analysis of variance[a] was performed, with time in days designated as the classification variable in order to estimate within-day and among-days components of variance and the variance of a single determination (sum of the estimates of "within" and "among" components). The estimated deviation (square root of variance) of a single determination and the approximate degrees of freedom[b] are listed in the table accompanying the appropriate figure for each pool (see figure 2, for example).

In addition to the information given in the tables on bench quality control data, the monthly means for each control pool are plotted to reduce and summarize available quality control data (see figure 2, for example).

Some pools were not used as bench controls; their data were analyzed in the runs in order to study various methods of preparing and storing pools. These data are not included in this summary report.

[a] Further details of quality control calculations are presented in section VI.D.

[b] Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

B. Individual Serum Laboratory Procedures and Quality Control Summaries

1. Serum Total Protein

Serum total protein is quantitated by measuring the intensity of the purple complex formed in the reaction between biuret reagent and protein at 550 nm in an adaptation of the Technicon AutoAnalyzer Methodology N-14b,[28] which is a modification of the biuret procedure proposed by Weichselbaum.[29]

Procedure.—0.05 ml of serum specimen, standard, or quality control sample is mixed with 2.5 ml of biuret reagent. The intensity of the resulting purple complex is read with a Beckman DB-GT Spectrophotometer at 550 nm in a 10-mm flowcell. The serum specimen blanks are measured at 550 nm with the biuret reagent replaced by alkaline potassium iodide solution. The corrected absorbance of the biuret-protein complex is obtained by subtracting the absorbance of the serum blank from the absorbance of the purple complex at 550 nm.

Total protein standards are prepared from a 10 g/dl solution of human albumin, fraction V. The following human albumin standards are analyzed in duplicate with every 20 serum specimens: 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 g/dl. Serum total protein concentrations are calculated from the slope of the standard curve, assuming zero intercept. Quality control samples are analyzed with each set of standards and with every 20 specimens. Total protein is reported as grams of protein per deciliter of serum (g/dl). The repeat limits are below 5.0 and above 8.0 g/dl.

The following are noted as modifications to the Technicon AutoAnalyzer Methodology N-14b[28]: (a) potassium tartrate is not included in the alkaline potassium iodide blank solution; (b) absorbances are measured with a spectrophotometer.

Monthly means for bench quality control pools for serum total protein with corresponding estimates of imprecision are shown in figure 2.

Estimates of imprecision: Serum total protein

Bench quality control						Quality surveillance							
						Pooled material					Sample resubmission[1]		
Pool number	Dates	Average (g/dl)	Estimated standard deviation (g/dl)	Coefficient of variation (%)	Approximate degrees of freedom[2]	Dates	Average (g/dl)	Estimated standard deviation (g/dl)	Coefficient of variation (%)	Degrees of freedom[2]	Concentration (g/dl)	Estimated standard deviation (g/dl)	Coefficient of variation (%)
9	Feb. 72 Feb. 73	7.07	0.304	4.3	590								
6	Feb. 72 Nov. 72	7.14	0.126	1.8	245								
1	May 71 Aug. 71	7.19	0.097	1.4	52								
5	Oct. 71 Jan. 72	7.39	0.135	1.8	59								
2	Aug. 71 Feb. 72	7.80	0.144	1.8	48								
											5.5	0.113	2.1
											6.0	0.124	2.1
						Oct. 73 Feb. 74	6.26	0.134	2.1	26	6.25	0.129	2.1
4	Feb. 74 July 74	6.42	0.104	1.6	37	Mar. 74 July 74	6.44	0.101	1.6	8	6.50	0.134	2.1
0	Jan. 73 Mar. 74	6.69	0.148	2.2	251						6.7	0.138	2.1
3	Jan. 73 July 74	7.11	0.173	2.4	218						7.0	0.144	2.1
											7.5	0.155	2.1

[1]Estimates of standard deviation based on 181 sample resubmissions between April 1973 and December 1973; 4 pairs (2.2%) were omitted from calculations.

[2]Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

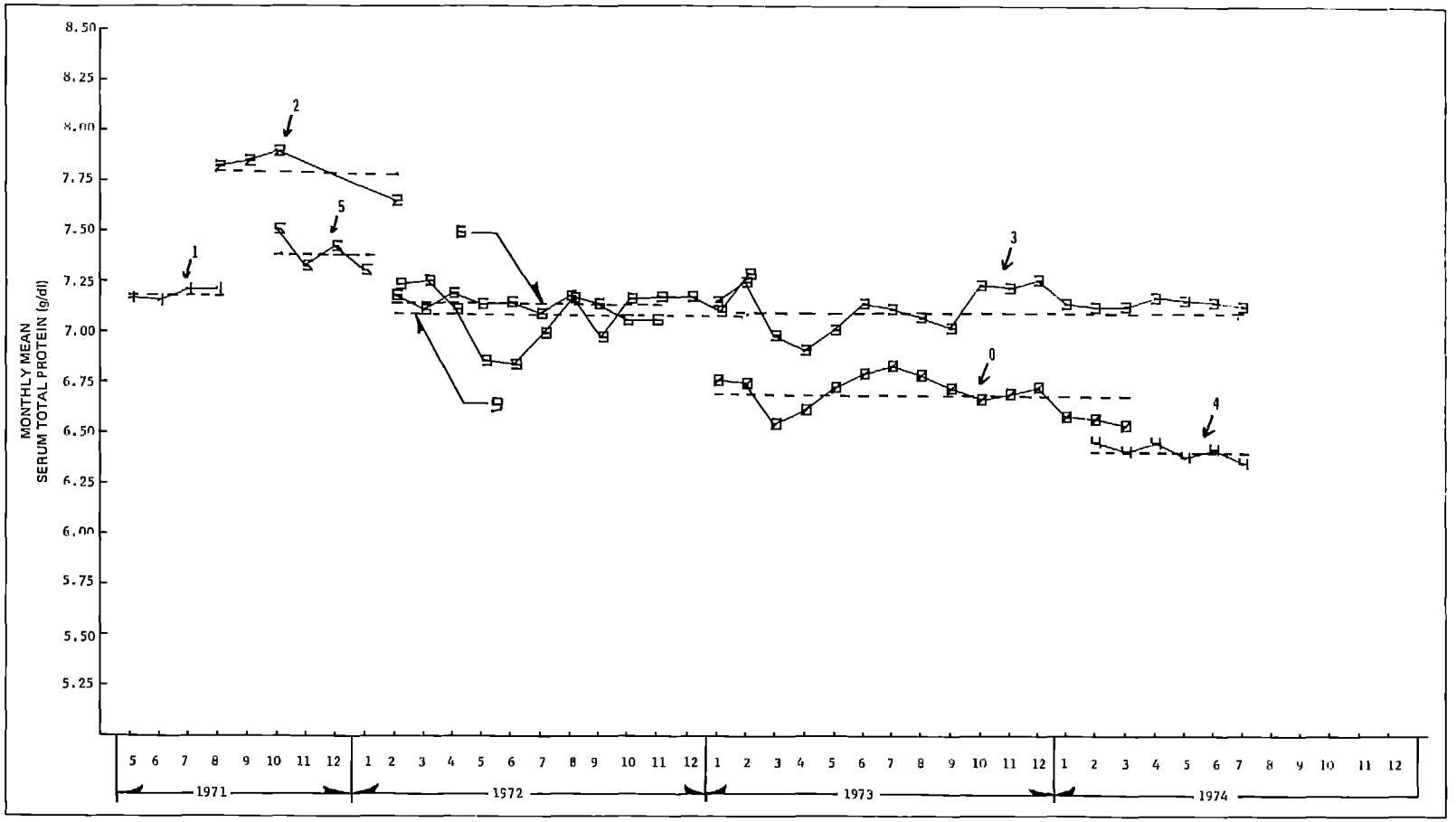

Figure 2. Monthly means for bench quality control pools: Serum total protein

2. Albumin

Serum albumin is quantitated by measuring the intensity of the green complex formed in the reaction between bromcresol green (BCG) and albumin in pH 4.2 buffer at 630 nm in a modification of the automated BCG method of Doumas et al.[30]

Procedure.—0.05 ml of serum specimen, standard, or quality control sample is diluted with 2.9 ml of saline. A 0.6-ml aliquot is mixed with 4.5 ml of BCG-succinate buffer solution. After an 8-minute reaction time, the absorbance of the BCG-albumin complex is measured with a Beckman DB-GT Spectrophotometer at 630 nm in a 10-mm flowcell.

Albumin standards are prepared from a 10 g/dl solution of human albumin, fraction V. The following human albumin standards are analyzed in duplicate with every 20 serum specimens: 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, and 8.0 g/dl. Serum albumin concentrations are calculated from the slope of the standard curve, assuming zero intercept. Quality control samples are analyzed with each set of standards and with every 20 specimens. Serum albumin is reported as grams of albumin per deciliter of serum (g/dl). Repeat limits are below 2.5 and above 5.5 g/dl.

The following are noted as modifications of the original method of Doumas et al.[30]: (a) the final concentration of the succinate buffer in working BCG solution is 0.071 mol/l; (b) the BCG concentration in working BCG solution is 0.012%; (c) the initial dilution of serum is made with 0.9% saline; (d) the final sample concentration is approximately three times more dilute than Doumas described; and (e) absorbances are measured with a spectrophotometer.

These particular reagent concentrations and their ratios are based on work conducted at CDC (unpublished). However, the CDC method is similar to the Technicon AutoAnalyzer II and SMA AutoAnalyzer Systems Bromcresol Green (BCG) Albumin Method, a description of which was published in 1973.[31]

Monthly means for bench quality control pools for serum albumin with corresponding estimates of imprecision are shown in figure 3.

Estimates of imprecision: Serum albumin

Bench quality control						Quality surveillance							
						Pooled material					Sample resubmission[1]		
Pool number	Dates	Average (g/dl)	Estimated standard deviation (g/dl)	Coefficient of variation (%)	Approximate degrees of freedom[2]	Dates	Average (g/dl)	Estimated standard deviation (g/dl)	Coefficient of variation (%)	Degrees of freedom[2]	Concentration (g/dl)	Estimated standard deviation (g/dl)	Coefficient of variation (%)
7	Feb. 72 Feb. 73	4.27	0.172	4.0	262								
6	July 72 Nov. 72	4.48	0.154	3.5	219								
5	Oct. 71 June 72	4.61	0.139	3.0	59								
1	May 71 Aug. 71	4.66	0.131	2.8	48								
2	Aug. 71 Feb. 72	4.94	0.101	2.0	67								
											3.0	0.114	3.8
											3.5	0.114	3.3
						Oct. 73 Feb. 74	4.0	0.122	3.0	27	4.0	0.114	2.9
0	Jan. 73 Mar. 74	4.30	0.108	2.5	286	Mar. 74 July 74	4.1	0.071	1.7	8	4.3	0.114	2.7
4	Feb. 74 June 74	4.37	0.105	2.4	36						4.4	0.114	2.6
3	Jan. 73 June 74	4.53	0.114	2.5	218						4.5	0.114	2.5
											5.0	0.114	2.3

[1] Estimates of standard deviation are based on 181 sample resubmissions between April 1973 and December 1973; 3 pairs (1.7%) were omitted from calculations.

[2] Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

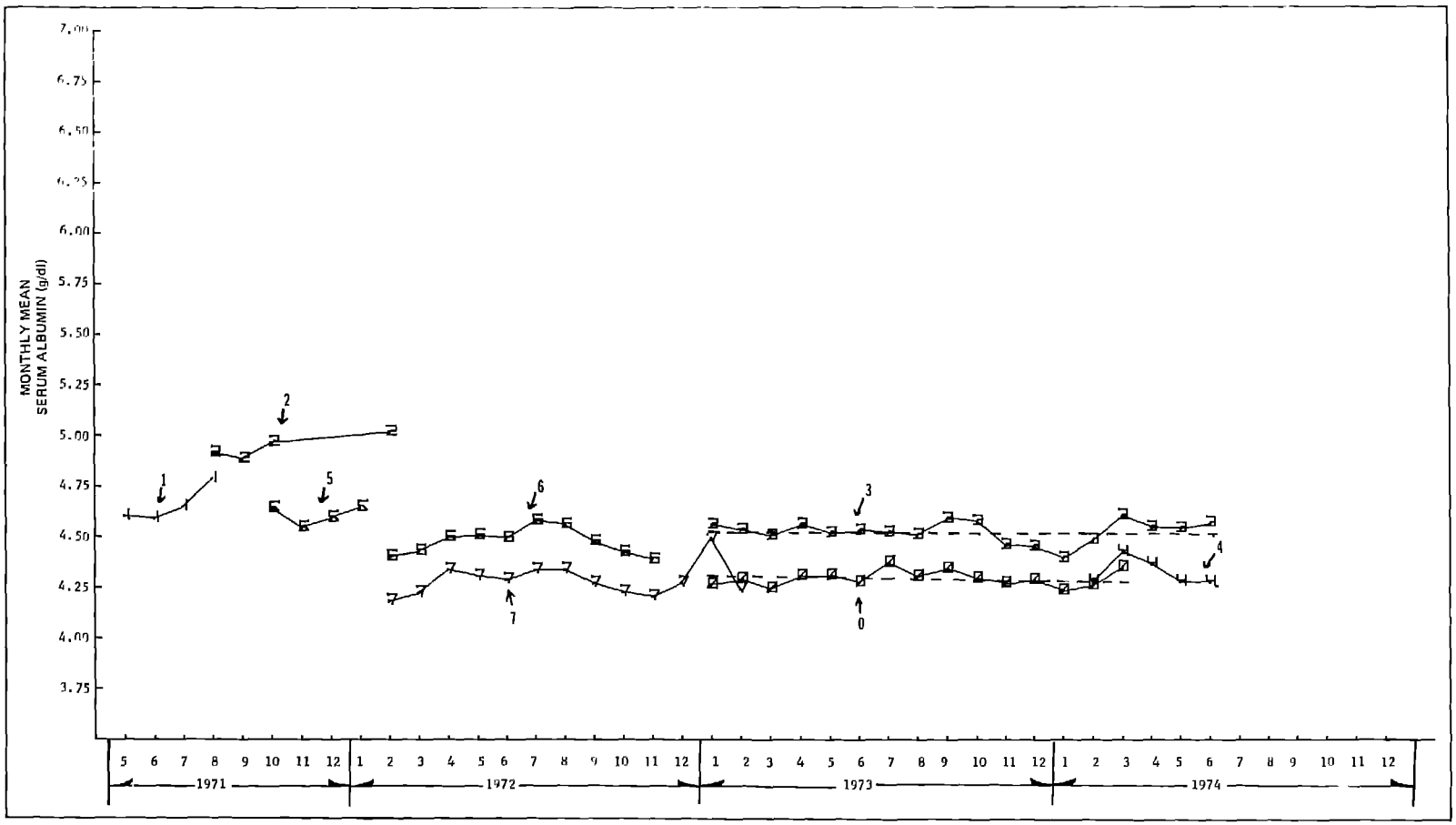

Figure 3. Monthly means for bench quality control pools: Serum albumin

3. Magnesium

Serum magnesium is measured by atomic absorption spectroscopy using the method of Hansen and Freier.[32] Instrumental parameters are those recommended in the Perkin-Elmer manual, *Analytical Methods for Atomic Absorption Spectroscopy.*[33]

Procedure.—Serum specimens, standards, and quality control samples are diluted 1:50 with distilled water. The diluted samples are aspirated into a flame, and the concentration of magnesium is measured. The Perkin-Elmer Model 306 Atomic Absorption Spectrophotometer is calibrated in concentration units with a 2-point calibration: 0.00 and 1.75 mEq/l. Magnesium standards are prepared from analytical grade magnesium turnings. The 1.75 mEq/l standard is analyzed with every 20 specimens to recheck the calibration. As a check on linearity, the entire set of standards (1.00, 1.25, 1.50, 1.75, 2.00, and 2.50 mEq/l) is analyzed periodically throughout the procedure. Quality control samples are analyzed with every 20 specimens. Serum magnesium is reported as milliequivalents of magnesium per liter of serum (mEq/l). Repeat limits are below 1.40 and above 2.10 mEq/l.

The following is noted as a modification of the original method of Hansen and Freier[32]: 5 mEq/l potassium and 140 mEq/l sodium are added to the standards to simulate the electrolyte concentration of human serum.

Monthly means for bench quality control pools for serum magnesium with corresponding estimates of imprecision are shown in figure 4.

Estimates of imprecision: Serum magnesium

Bench quality control					
Pool number	Dates	Average (mEq/l)	Estimated standard deviation (mEq/l)	Coefficient of variation (%)	Approximate degrees of freedom[1]
1	Feb. 72 Mar. 72	1.713	0.0258	1.51	58
2	Dec. 71 Mar. 73	1.642	0.0249	1.52	201
3	May 73 Apr. 75	1.698	0.0331	1.95	311
8	Aug. 74 Sept. 75	1.973	0.0457	2.32	22
9	Aug. 74 Sept. 75	1.011	0.0302	2.98	20

[1]Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

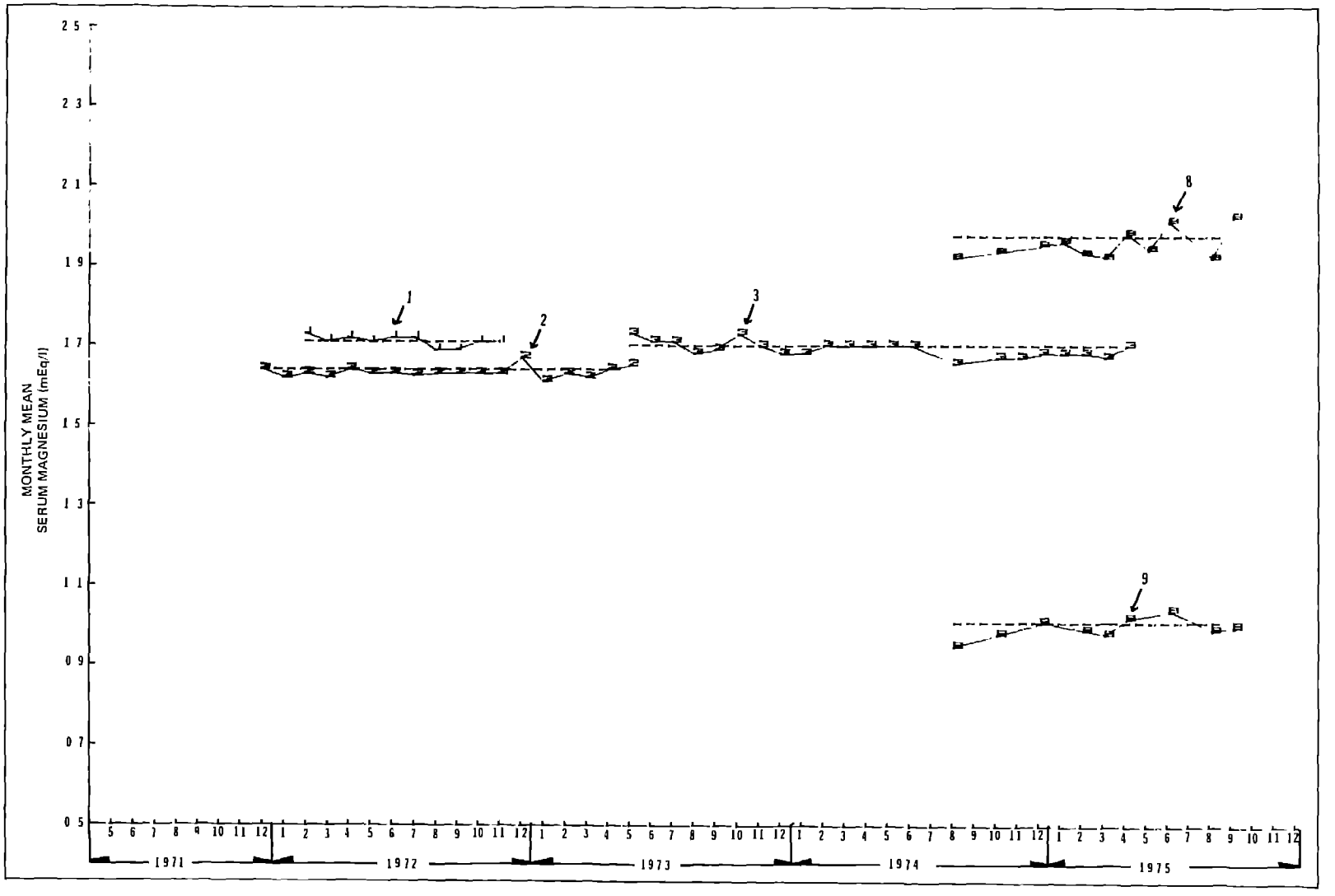

Figure 4. Monthly means for bench quality control pools: Serum magnesium

4. Folic Acid

Serum folic acid is measured microbiologically by a semiautomated modification of the methods described by Baker et al.[34] and Cooperman.[35] An aliquot of serum extract is added to an assay medium containing all the nutrients except folic acid that are necessary for the growth of *Lactobacillus casei* (ATCC 7469). The medium is then inoculated with this microorganism and incubated for 20-22 hours at 37° C. Because the growth of the *L. casei* is proportional to the amount of folic acid present in the serum extract, the folic acid level can be quantitated by measuring the turbidity of the inoculated medium.

Procedure.—0.4 ml of serum specimen or quality control sample is diluted with 3.6 ml, pH 6.1, phosphate buffer containing 1 mg/ml ascorbic acid. The serum-buffer mixtures are autoclaved for 3 minutes at 121° C, 15 psi, to precipitate serum proteins. Samples are cooled and centrifuged at 1,500 rev/min for 10 minutes and the supernatant is decanted. A 0.1-ml aliquot of each protein-free supernatant is mixed with 1.9 ml of assay medium, and all the assay tubes are autoclaved for 3 minutes at 121° C, 15 psi. Each assay tube is then inoculated with 1 drop of a 1:100 dilution of a 6-hour *L. casei* culture prepared from an 18-hour broth culture. The assay tubes are incubated in a 37° C circulating water bath for 20-22 hours, and then removed and placed at 4° C for at least 15 minutes to inhibit further growth. Assay tubes are vortexed to resuspend the *L. casei,* and the turbidity of samples is measured as absorbance at 600 nm in a 15-mm flowcell with a Technicon AutoAnalyzer Colorimeter.

Standards for the folic acid assay are prepared from analytical grade pteroylglutamic acid (PGA). The following concentrations of PGA standards are analyzed in triplicate with every 100-120 serum specimens: 1.0, 2.0, 4.0, 6.0, 8.0, 10.0, 12.0, 14.0, 16.0, 18.0, and 20.0 ng/ml. A nonlinear standard curve is obtained by plotting the absorbance of standards against PGA concentration. Serum folic acid concentrations are read directly from the standard curve. Quality control samples are analyzed in triplicate with every 100-120 serum specimens. Because of the inherent variability in microbiological assays, each protein-free filtrate is analyzed in duplicate. Folate concentrations are reported as nanograms of PGA per milliliter of serum (ng/ml). Repeat limits are below 2.0 and above 18.0 ng/ml.

The following are noted as modifications in the methods of Baker et al.[34] and Cooperman:[35] (a) The automated turbidity measurement is described by Slade et al.,[36] (b) assay medium is prepared according to the formula described by Baker et al.,[34] and (c) the concentrations of standards differ from those described by Baker et al.[34] and Cooperman (1967).[35]

Monthly means for bench quality control pools for serum folic acid with corresponding estimates of imprecision are shown in figure 5.

Estimates of imprecision: Serum folic acid

Bench quality control

Pool number	Dates	Average (ng/ml)	Estimated standard deviation (ng/ml)	Coefficient of variation (%)	Approximate degrees of freedom[1]
1	Aug. 73 Sept. 75	5.5	0.85	15.30	134
2	Aug. 73 Oct. 75	3.7	0.51	13.80	127
3	Aug. 73 Oct. 75	2.7	0.47	17.50	137
4	Aug. 73 Oct. 75	6.0	1.01	16.60	124
5	Mar. 74 Oct. 75	4.2	0.57	13.30	90

[1]Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

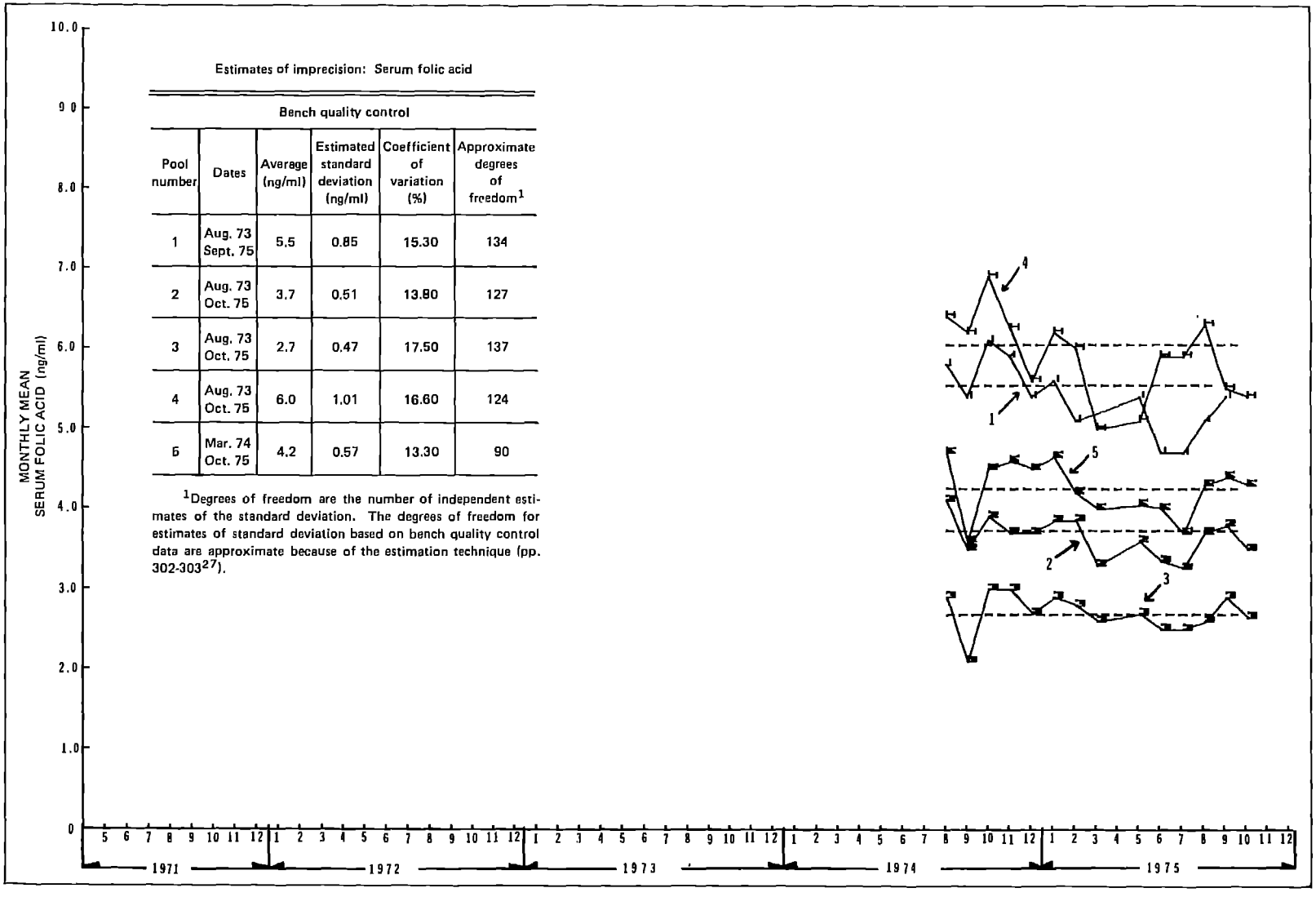

Figure 5. Monthly means for bench quality control pools: Serum folic acid

5. Vitamin A

Vitamin A is quantitated by measuring the intensity of the transient blue color formed in the reaction between trifluoroacetic acid (TFA) and vitamin A at 620 nm by the method of Neeld and Pearson.[37]

Procedure.—2 ml of serum specimen or quality control sample is shaken with 2 ml of 95% alcohol to precipitate the proteins, and with 3 ml of petroleum ether to extract the vitamin A. A 2-ml aliquot of the petroleum-ether phase of each supernate is pipetted into a 10 X 75-mm cuvette. The absorbance of the β-carotene is read with a Coleman Jr. Spectrophotometer at 450 nm. Then the petroleum ether is evaporated under a stream of dry nitrogen at 50° C. The residue is dissolved in 0.1 ml chloroform. The cuvette is placed in a Coleman Jr. Spectrophotometer at 620 nm; 1 ml chromogen (1 part TFA and 2 parts chloroform) is added to the cuvette; and the absorbance of the transient blue color at 620 nm is registered immediately on a strip chart recorder.

The contribution of the β-carotene to the absorbance at 620 nm is determined from its absorbance at 450 nm using a factor calculated according to the procedure described by Neeld and Pearson.[37] The absorbance of β-carotene at 620 nm is subtracted from the total absorbance at 620 nm to give the corrected absorbance of the vitamin A-TFA complex.

Vitamin A standards are prepared from all trans-retinyl acetate (vitamin A acetate). The following concentrations of vitamin A standards are prepared in duplicate: 40, 80, 120, 160, 200, and 250 μg/dl. β-carotene standards are prepared from trans-β-carotene. The following concentrations of β-carotene standards are used: 40, 80, 160, and 320 μg/dl. Both vitamin A and β-carotene standards are unstable and must be prepared on the day they are used. Both sets of standards are periodically analyzed in duplicate to verify the derived mathematical factors. Serum vitamin A concentrations are calculated from the slope and y-intercept of the vitamin A acetate standard curve. These values are converted to vitamin A alcohol values by multiplying the vitamin A acetate values by 0.872. Vitamin A is reported as micrograms of vitamin A alcohol per deciliter of serum (μg/dl).

Quality control samples are analyzed with every 20 specimens. Repeat limits are below 20 and above 150 μg/dl.

The following are noted as modifications of the original Neeld and Pearson procedure:[37] (a) KOH is omitted from the 95% ethanol; (b) petroleum ether is evaporated at 50° C; (c) acetic anhydride is not used; (d) absorbance readings at 620 nm are obtained at the "pause point," approximately 10 seconds after chromogen is added; and (e) β-carotene and vitamin A acetate standards differ in concentration from those used in the original method, but include the same concentration range.

Monthly means for bench quality control pools for serum vitamin A with corresponding estimates of imprecision are shown in figure 6.

Estimates of imprecision: Serum vitamin A

Bench quality control

Pool number	Dates	Average (μg/dl)	Estimated standard deviation (μg/dl)	Coefficient of variation (%)	Approximate degrees of freedom[1]
1	May 71 Apr. 73	53	7 1	13 4	360
6	Feb. 74 June 74	58	4.5	7.8	73
5	Jan. 73 Mar. 74	61	4.9	8.0	248

[1]Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

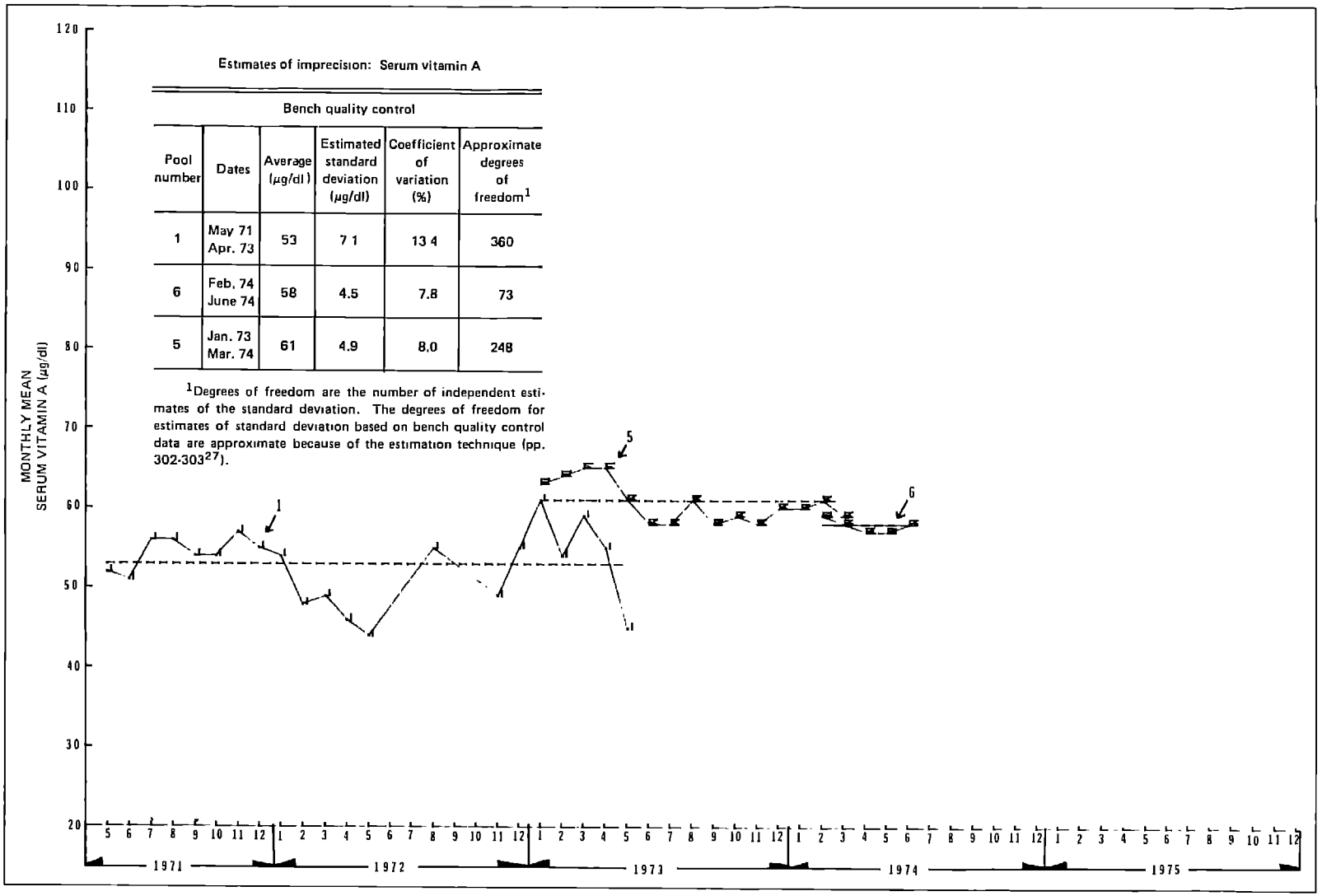

Figure 6. Monthly means for bench quality control pools: Serum vitamin A

6. Iron and Total Iron-binding Capacity

Serum iron and total iron-binding capacity (TIBC) are determined by a modification of the automated Technicon AAII-25 Method,[38] which is based on the procedure of Giovanniello et al.[39] and Ramsey.[40] Iron is quantitated by measuring the intensity of the violet-colored complex formed in the reaction between Ferrozine and Fe(II) in pH 5.0 buffer at 562 nm.

Procedure.—0.8 ml of serum specimen, standard, or quality control sample is mixed with 1.2 ml of diluent containing ascorbic acid and sodium chloride in 0.2 mol/l HCl. The iron in the acidified mixture is dialyzed into a solution of 0.2 mol/l HCl and sodium chloride. The dialysate is mixed with 1.6 ml of 0.7% Ferrozine in pH 5.0 acetate buffer. The absorbance of the Ferrozine-Fe(II) complex is measured on a colorimeter at 570 nm in a 50-mm flowcell.

To determine TIBC, the serum specimens and quality control samples are diluted 1:3 with 400 μg/dl iron-saturating solution to saturate the iron-binding sites of the serum transferrin. Approximately 0.2 g light magnesium carbonate is used to remove excess iron. After it is centrifuged, the supernatant is analyzed for iron by the serum iron method.

Iron standards are prepared from analytical grade iron wire. The following concentrations of iron standards are analyzed with every 20-30 serum iron or TIBC specimens: 30, 50, 80, 100, 150, 200, 250, and 200 μg/dl. Iron concentrations of serum specimens and diluted TIBC samples are calculated from the slope and y-intercept of the standard curve. The total iron-binding capacity of a serum specimen is calculated by multiplying the iron concentration in the diluted TIBC sample by 3, the dilution factor. Quality control samples are analyzed in duplicate with each set of standards and with every 20 specimens. Serum iron and TIBC are reported as micrograms of iron per deciliter of serum (μg/dl). Repeat limits for serum iron are below 50 and above 250 μg/dl. Repeat limits for TIBC are below 250 and above 500 μg/dl.

The following are noted as modifications to the Automated Technicon AAII-25 Method:[38] (a) The reagent concentrations and their ratios are based on unpublished procedures developed at CDC, (b) two standard Technicon AutoAnalyzer I dialysis plate assemblies with type-C membranes are connected in series to increase the efficiency of dialysis, (c) Ferrozine is incorporated into the acetate buffer, and (d) a 50-mm flowcell is used.

Monthly means for bench quality control pools for serum iron and total iron-binding capacity with corresponding estimates of imprecision are shown in figures 7 and 8.

Errata: Serum iron and transferrin saturation.—Following the publication of the "Preliminary Findings of the First Health and Nutrition Examination Survey, United States, 1971-1972: Dietary Intake and Biochemical Findings,"[41] a different analytical method for measuring serum iron and total iron-binding capacity (just described) was adopted for the remainder of HANES I. Although based on the same analytical principles applied in the original method of White and Flaschka,[42] the modified method includes a dialysis procedure. A comparison study of the original and AutoAnalyzer methods revealed unacceptable variability in the iron and total iron-binding capacity results obtained with the original method. Serum specimens for a major portion of HANES examinees were taken from a reserve vial collection stored at -20° C and were reanalyzed by the AutoAnalyzer method between December 1974 and May 1975. The quality control summaries for the analytes included in this report contain only data obtained with the AutoAnalyzer method. These serum iron and transferrin saturation results supersede all previously published results.[43]

Estimates of imprecision: Serum iron

Bench quality control

Pool number	Dates	Average (μg/dl)	Estimated standard deviation (μg/dl)	Coefficient of variation (%)	Approximate degrees of freedom[1]
3	July 73 Dec. 74	107.4	2.77	2.60	179
7	Apr. 75 May 75	94.9	2.04	2.10	577
8	Apr. 75 May 75	61.9	2.69	4.40	366

[1]Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

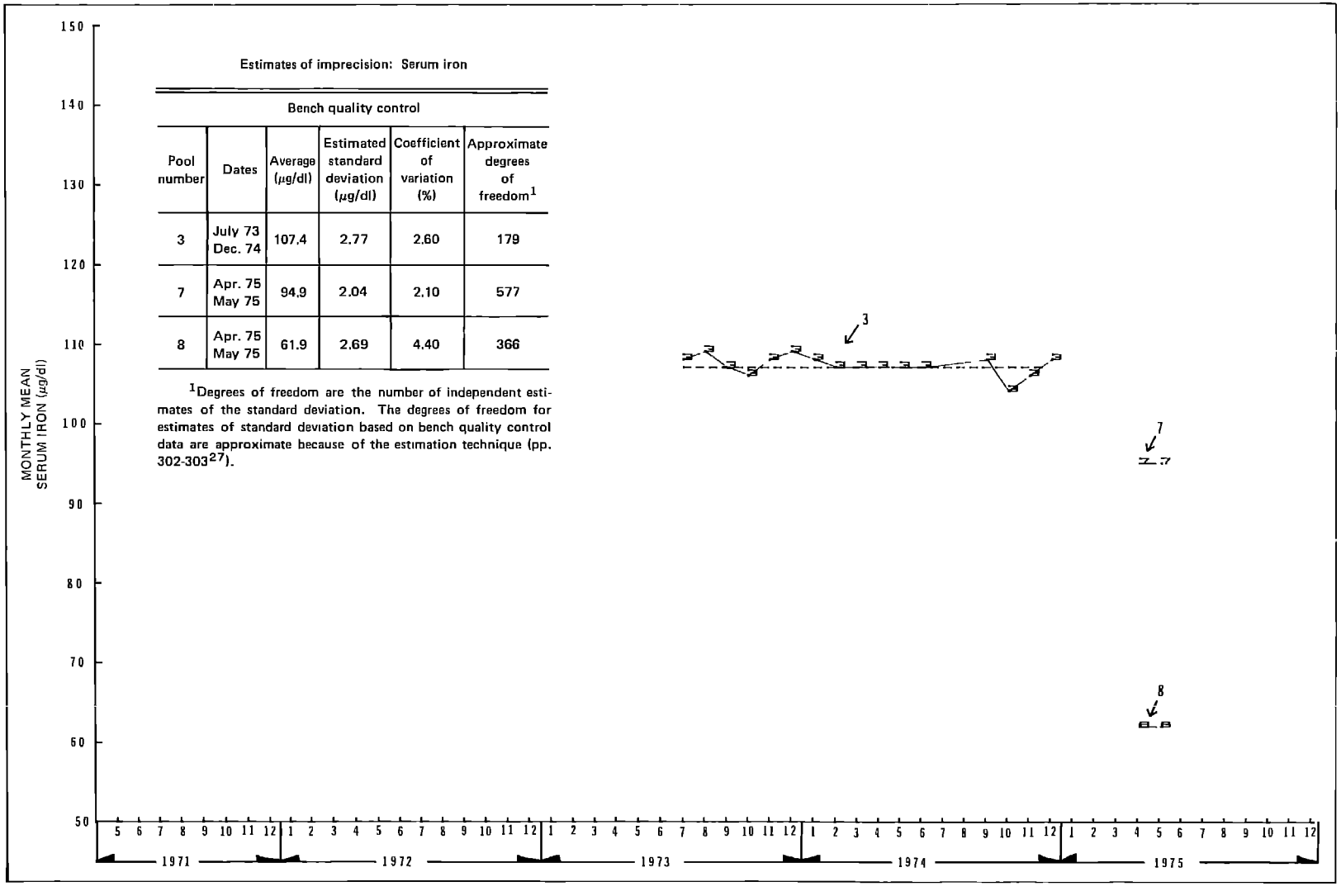

Figure 7. Monthly means for bench quality control pools: Serum iron

Estimates of imprecision: Serum total iron-binding capacity

Bench quality control					
Pool number	Dates	Average (μg/dl)	Estimated standard deviation (μg/dl)	Coefficient of variation (%)	Approximate degrees of freedom[1]
3	July 73 Feb. 75	365.0	14.91	4.10	528
7	Dec. 74 Feb. 75	374.7	13.09	3.50	171

[1]Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

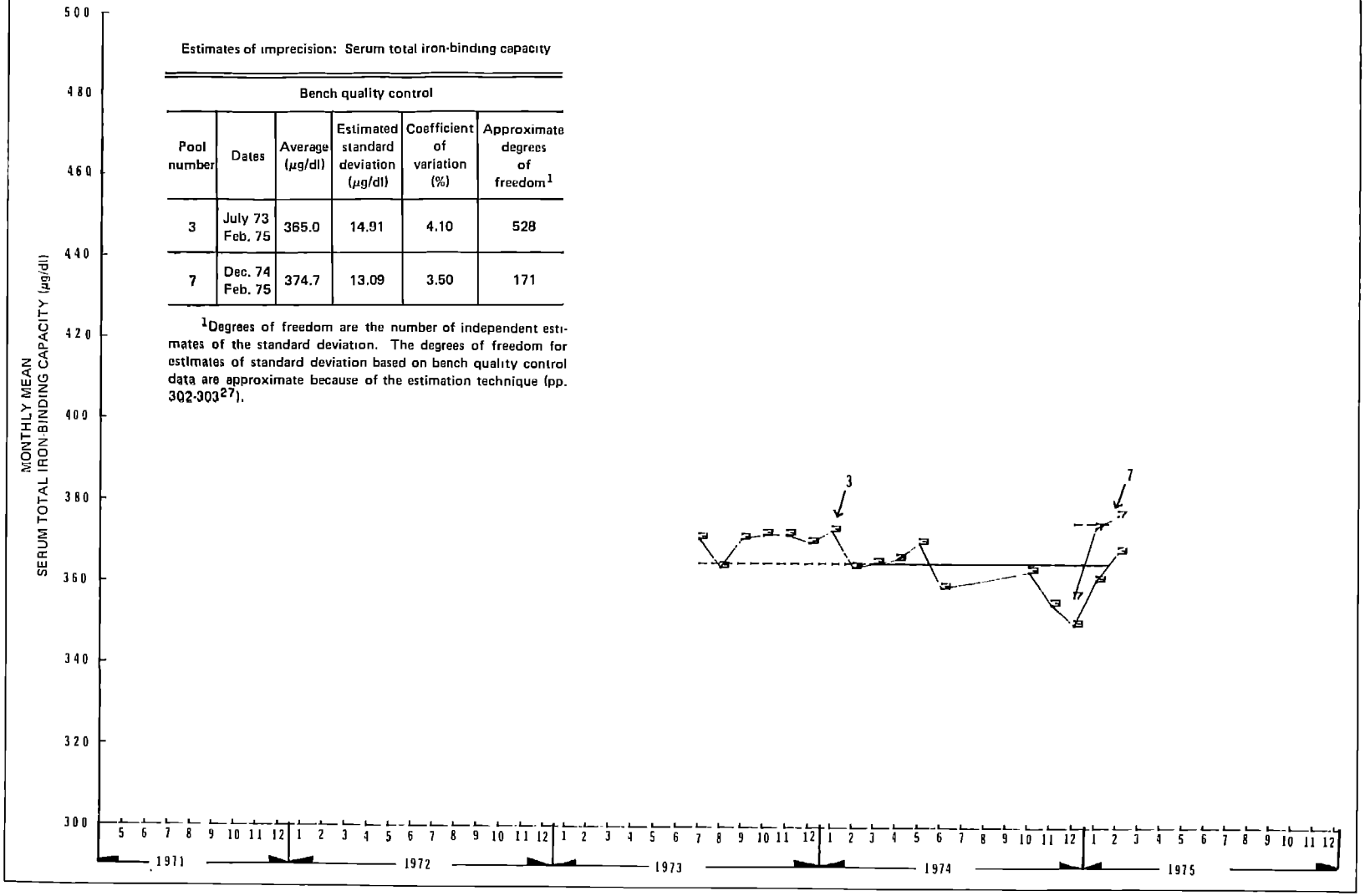

Figure 8. Monthly means for bench quality control pools: Serum total iron-binding capacity

7. Serum Glutamic-Oxalacetic Transaminase (SGOT)

Serum glutamic-oxalacetic transaminase (SGOT) is determined by an automated modification of the method of Henry et al.[44] In this method the following two reactions are coupled:

$$\text{Aspartic acid} + \alpha\text{-ketoglutaric acid} \xrightarrow{\text{SGOT}} \text{oxalacetic acid} + \text{glutamic acid.}$$

$$\text{Oxalacetic acid} + \text{NADH} + \text{H}^+ \xrightarrow{\text{MDH}} \text{malic acid} + \text{NAD.}$$

As soon as oxalacetic acid is formed by the transaminase reaction, it is reduced to malic acid as an equivalent amount of NADH is oxidized to NAD^+. SGOT is determined indirectly by measuring the decrease in absorbance of NADH at 340 nm.

Procedure.—This SGOT procedure is performed on an Electro-Nucleonics GemSAEC Centrifugal Fast Analyzer. 50 μl of serum specimens or quality control samples are diluted with 400 μl reagent containing phosphate buffer, aspartic acid, NADH, and malic dehydrogenase (MDH). The diluted samples are placed in the outer wells of a 16-well analytical rotor, and 50 μl of α-ketoglutaric acid substrate is added to each of the adjacent reagent wells of the rotor. Samples and reagents are then incubated for 15 minutes at 30° C. After incubation, the reaction is initiated by mixing the contents of each pair of wells by centrifuging. The absorbance of the NADH is read 46 seconds after the reaction starts and every 60 seconds thereafter for 5 minutes. The rate of the reaction, that is, the change in absorbance per minute (ΔA/min), is calculated for each sample for the six absorbance readings.

The concentrations of enzymes are proportional to their enzymatic activity in a given reaction. The Karmen unit, used for reporting SGOT, is defined as the quantity of enzyme in 1.0 ml serum that will cause a change in the absorbance of NADH of 0.001 unit/min at a specified temperature. SGOT concentrations are calculated from the average ΔA/min as follows:

$$\text{SGOT (Karmen units/ml)} = \frac{\text{average } \Delta A/\text{min}}{\text{ml serum}} \times 1{,}000$$

Quality control samples are analyzed before any serum specimens and with every 13 serum specimens. SGOT is reported as Karmen units/ml of serum at 28° C. Repeat limits are below 5.0 and above 70 Karmen units/ml.

The following is noted as a modification of the original method of Henry et al.:[44] The concentrations of reagents and their exact ratios are similar to those recommended by Henry et al. and are the result of procedures performed at CDC.

Initially, SGOT was analyzed by the Technicon SMA 12/60 SGOT 340 nm method. The SMA 12/60 method is an automated photometric method described by Rush et al.,[45] who adapted it from the fluorometric method of Levine and Hill.[46] Because of technical difficulties with the SGOT channel, the SGOT method was transferred to the LKB Reaction Rate Analyzer and later to the Electronucleonics GemSAEC Centrifugal Fast Analyzer. In principle, each of these procedures is based on the original method of Henry et al.,[43,44] but the analyses were conducted at different

temperatures from those originally described. The results from each method were converted to Karmen units at 28° C.

Monthly means for bench quality control pools for SGOT with corresponding estimates of imprecision are shown in figure 9.

Estimates of imprecision: Serum glutamic-oxalacetic transaminase (SGOT)

Bench quality control					
Pool number	Dates	Average (Karmen units/ml)	Estimated standard deviation (Karmen units/ml)	Coefficient of variation (%)	Approximate degrees of freedom[1]
9	Jan. 72 June 72	218.6	15.29	7.00	11
11	Oct. 71 June 72	19.3	3.68	19.10	25
73	Mar. 72 June 72	20.5	2.70	13.20	13
83	Jan. 72 June 72	163.1	10.32	6.30	7
52	July 72 June 74	20.7	1.48	7.20	64
55	July 72 June 74	44.0	3.09	7.00	44
58	July 72 June 74	111.3	3.31	3.00	46
50	July 72 May 74	236.1	5.64	2.40	48
1	Aug. 74 Sept. 75	12.1	1.11	9.20	132
2	Aug. 74 Apr. 75	34.0	1.95	5.70	63
3	Aug. 74 Apr. 75	71.5	3.34	4.70	50
4	Aug. 74 Sept. 75	132.5	6.30	4.80	91
5	Aug. 74 Nov. 75	22.5	4.29	19.10	195
6	Aug. 74 Nov. 75	108.9	5.34	4.90	79

[1]Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

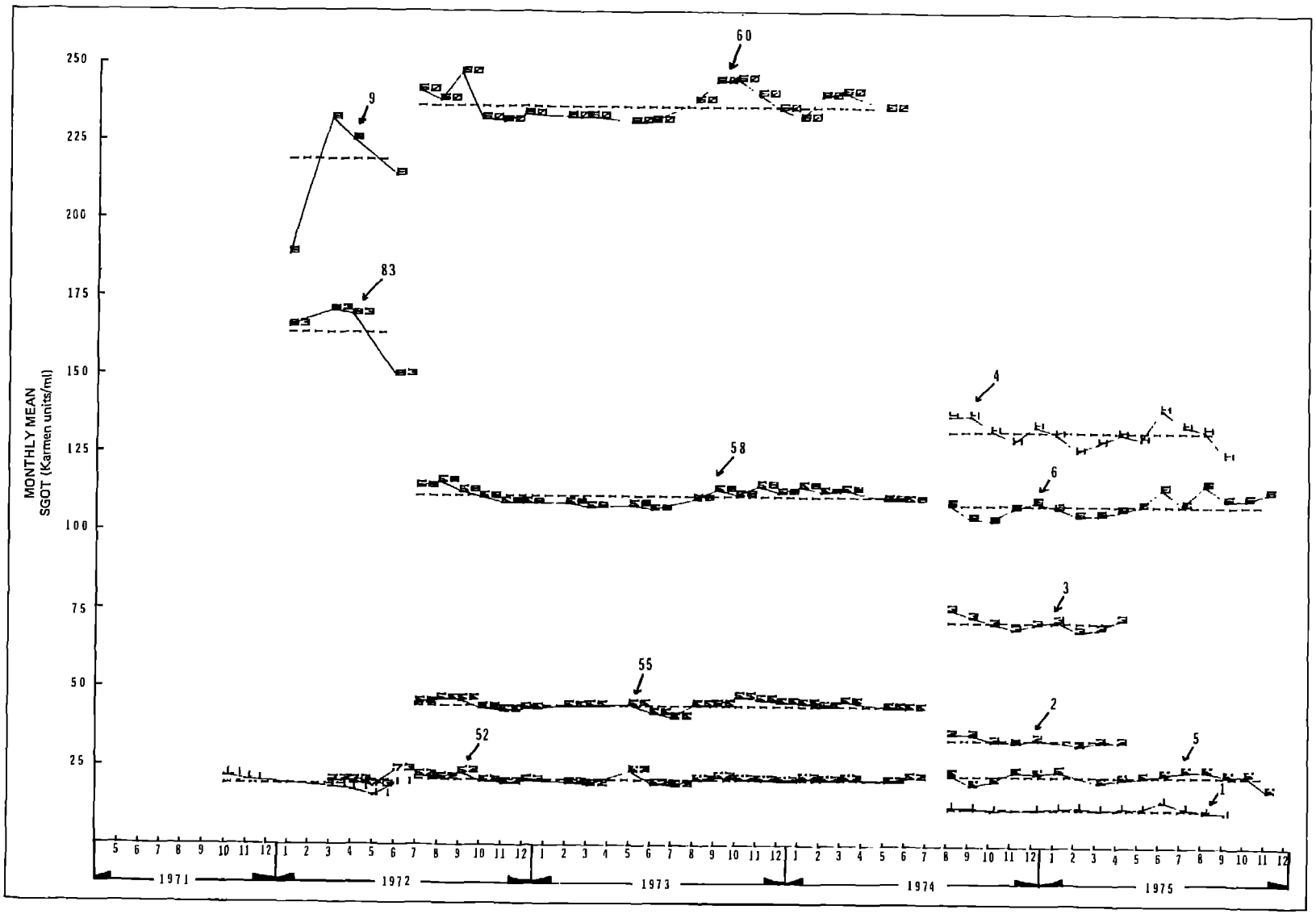

Figure 9. Monthly means for bench quality control pools: Serum glutamic-oxalacetic transaminase (SGOT)

8. Sodium and Potassium

Serum sodium and potassium are measured simultaneously by flame photometry by the method recommended in the Instrumentation Laboratory's *Operator's Manual IL 143 Digital Flame Photometer.*[47]

Procedure.—Serum specimens, standards, and quality control samples are diluted 1:200 with 15 mEq/l lithium diluent. The diluted samples are aspirated into a flame, and the sodium and potassium concentrations are read. The Instrumentation Laboratory Model 143 Digital Flame Photometer is calibrated with a diluent blank and a 140/4 mEq/l sodium/potassium (Na/K) standard. Sodium and potassium standards are prepared from National Bureau of Standards Reference Material NaCl and KCl. The 140/4 mEq/l Na/K standard is analyzed with every five specimens to check calibration. Quality control samples are analyzed with every five specimens. Sodium and potassium are reported as milliequivalents of sodium or potassium per liter of serum (mEq/l). Repeat limits for sodium are below 130 and above 150 mEq/l, and those for potassium are below 3.0 and above 5.0 mEq/l.

The following are noted as modifications of the method recommended by Instrumentation Laboratory, Inc.:[47] (a) When the specimen concentration is less than the lower repeat limits, the instrument is recalibrated with a 120/2 mEq/l Na/K standard and the specimen is reanalyzed; (b) when the specimen concentration is greater than the upper repeat limits, the instrument is recalibrated with a 150/6 mEq/l Na/K standard and the specimen is reanalyzed.

Monthly means for bench quality control pools for serum sodium and potassium with corresponding estimates of imprecision are shown in figures 10 and 11.

Estimates of imprecision: Serum sodium

Bench quality control					
Pool number	Dates	Average (mEq/l)	Estimated standard deviation (mEq/l)	Coefficient of variation (%)	Approximate degrees of freedom[1]
7	Aug. 74 Oct. 75	159.9	1.10	0.69	157
8	Aug. 74 Oct. 75	140.3	0.92	0.65	348
9	Aug. 74 Oct. 75	120.2	0.89	0.74	137

[1]Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

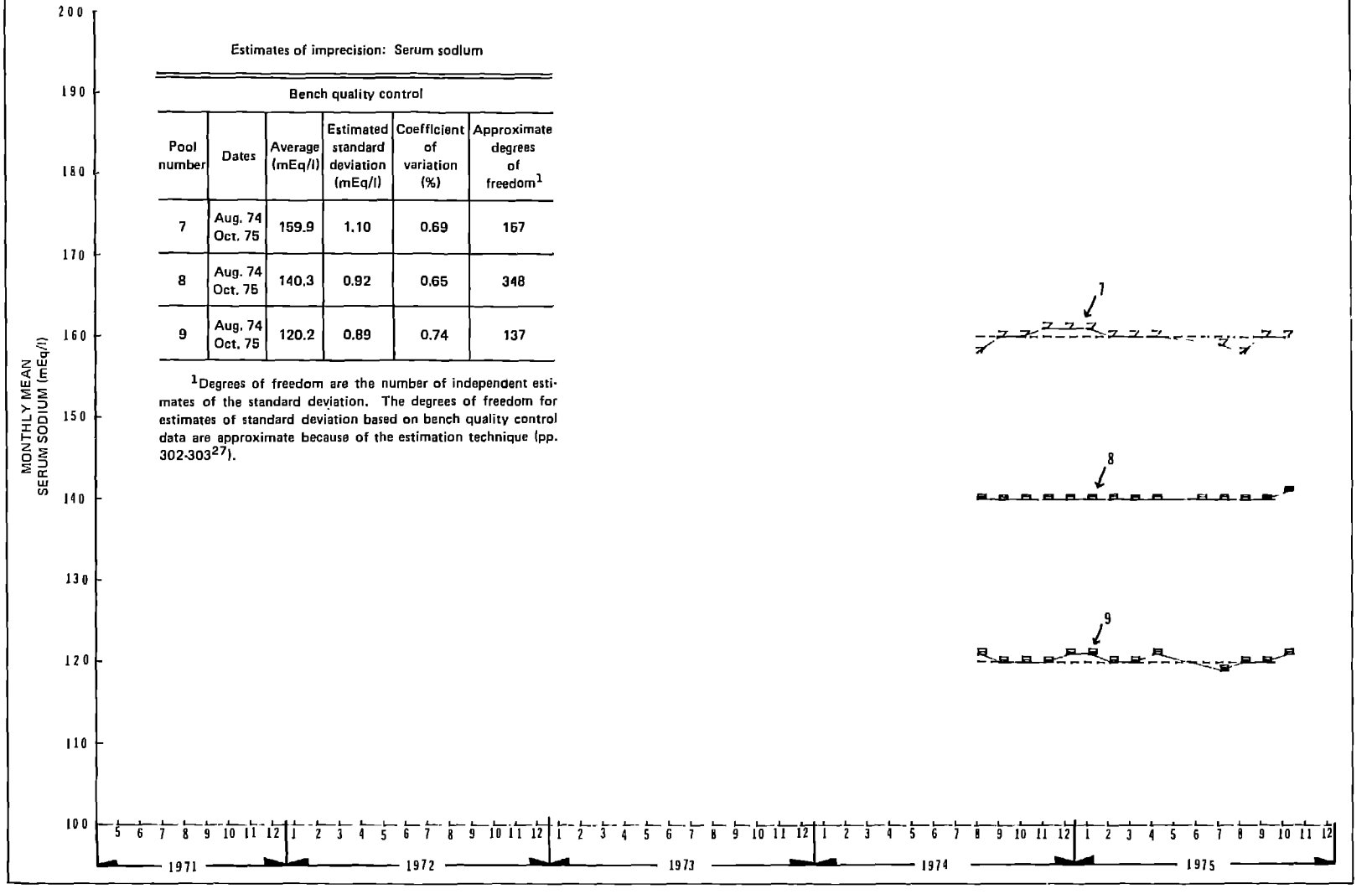

Figure 10. Monthly means for bench quality control pools: Serum sodium

Estimates of imprecision: Serum potassium

Bench quality control

Pool number	Dates	Average (mEq/l)	Estimated standard deviation (mEq/l)	Coefficient of variation (%)	Approximate degrees of freedom[1]
7	Aug. 74 Oct. 75	6.08	0.084	1.40	198
8	Aug. 74 Oct. 75	4.18	0.073	1.80	466
9	Aug. 74 Oct. 75	2.30	0.053	2.30	113

[1]Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

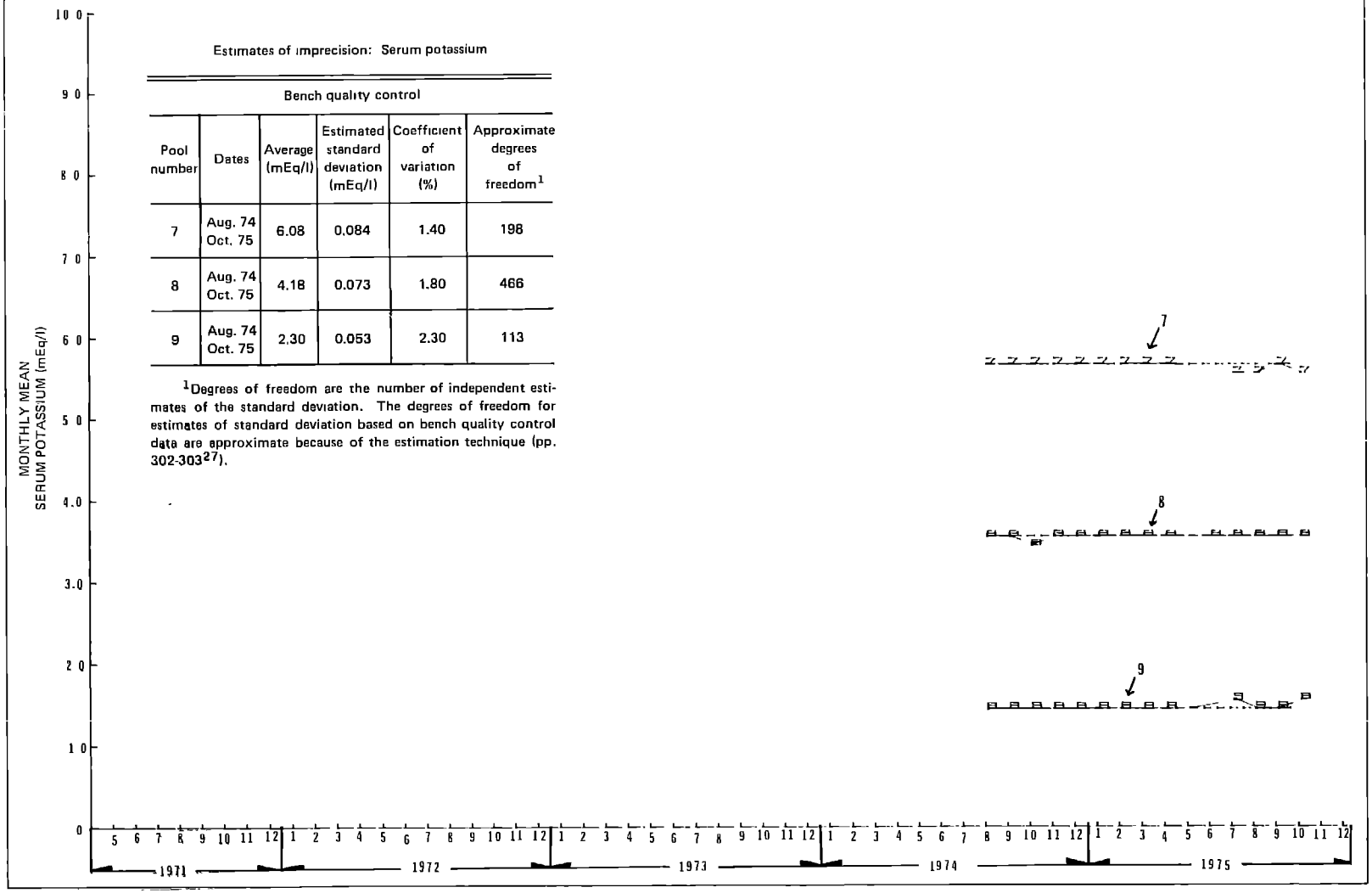

Figure 11. Monthly means for bench quality control pools: Serum potassium

9. Cholesterol

Serum cholesterol is determined by measuring the intensity of the green complex formed in the Liebermann-Burchard reaction at 620 nm, using the Eavenson et al.[48] modification of the Abell-Kendall method[49] which includes: (a) extraction of cholesterol, (b) saponification of esters to destroy nonspecific chromogens, and (c) color development. Acetic acid and acetic anhydride convert cholesterol to bis-3,5-cholestadiene, which reacts with sulfuric acid to form bis-cholestadienyl-monosulfonic acid.

Procedure.—0.1 ml of serum specimen, standard, or quality control sample is added to 1 ml of alcoholic potassium hydroxide in a glass tube. This saponification mixture is incubated at 45° C for 60 minutes. After the tubes are cooled to 25° C, 1.0 ml of distilled water and 2.0 ml of petroleum ether are added to each tube. The tubes are shaken for 5 minutes, and 1 ml of the petroleum ether phase is removed; 2 ml of petroleum ether is added. The solvent is removed by evaporation at 40-50° C in a vacuum oven.

2.0 ml of Liebermann reagent (acetic anhydride sulfuric acid glacial acetic acid) is added sequentially at 15-second intervals to each tube, which is then placed in a water bath to reach 25° C uniformly. The absorbance of each sample is read against the Liebermann reagent blank in a spectrophotometer at 620 nm exactly 30 minutes after the color reagent is added.

Five standard solutions of purified cholesterol are prepared in absolute alcohol at levels of 50, 100, 200, 300, and 400 mg/dl. Serum cholesterol concentrations are calculated from the slope and the y-intercept of the standard curve. All samples are analyzed in duplicate, with standards and quality control materials randomized throughout the run. Five levels of quality control materials are used; one reagent blank is prepared for every 45 samples.

Serum cholesterol is reported as milligrams of cholesterol per deciliter of serum (mg/dl). Repeat limits are below 150 and above 450 mg/dl.

The following are noted as modifications of the Eavenson procedure: (a) a Micromedic Automatic Pipette Model 2500 is used for all sampling and dispensing steps, (b) a Spectronic 100 Spectrophotometer equipped with a flow-through sampling system and a digital printer is used, and (c) the number of samples assayed per day is increased from 50 to 100 duplicates.

Monthly means for bench quality control pools for serum cholesterol with corresponding estimates of imprecision are shown in figure 12.

Estimates of imprecision: Serum cholesterol

Bench quality control

Pool number	Dates	Average (mg/dl)	Estimated standard deviation (mg/dl)	Coefficient of variation (%)	Approximate degrees of freedom[1]
1	July 71 Jan. 72	177.3	3.20	1.80	62
2	Nov. 72 Oct. 75	156.9	3.25	2.07	225
3	Nov. 72 Oct. 75	255.1	3.11	1.22	219

[1]Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data *are approximate* because of the estimation technique (pp. 302-303[27]).

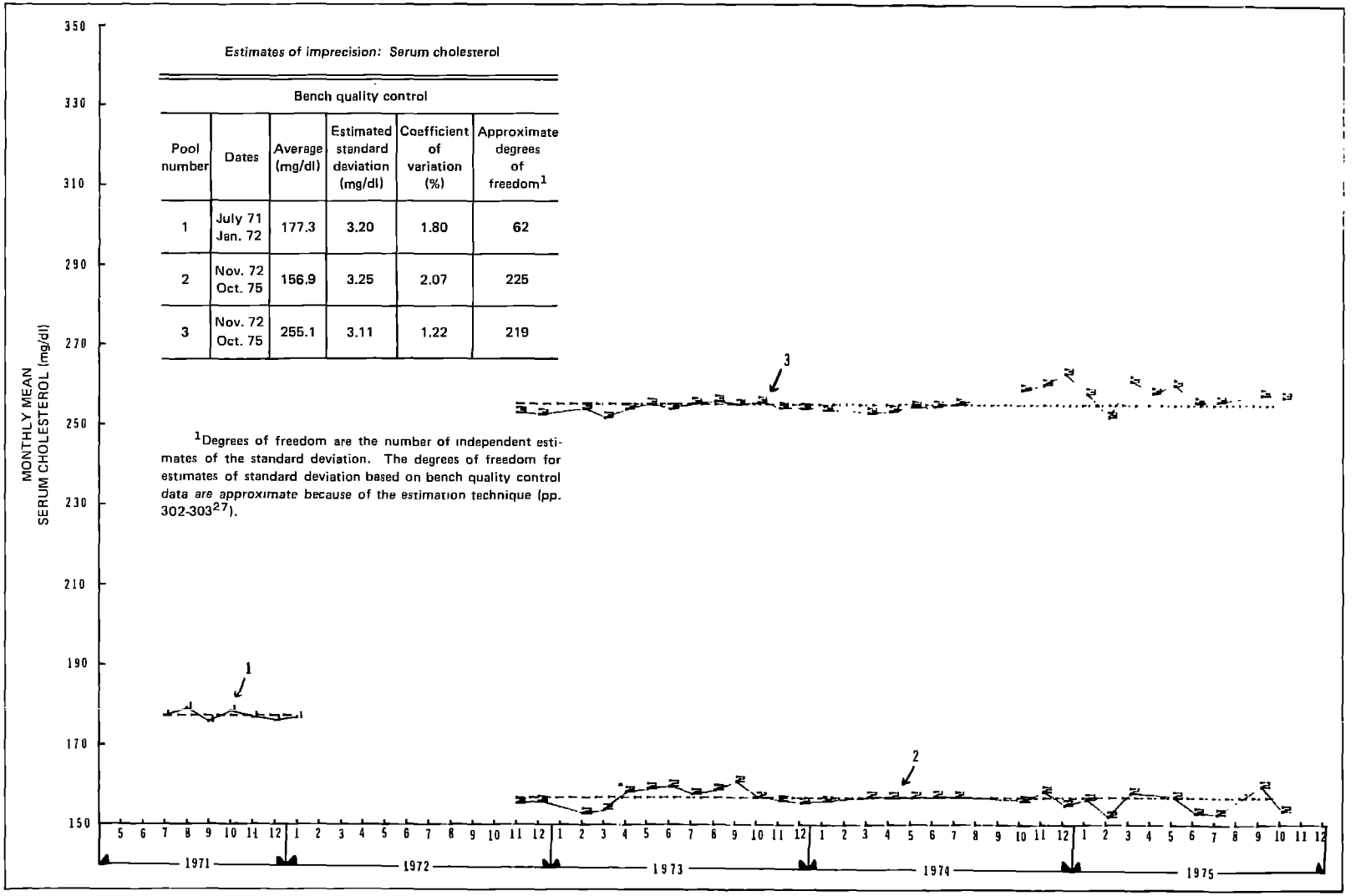

Figure 12. Monthly means for bench quality control pools: Serum cholesterol

C. Sequential Multiple Analyzer (SMA) 12/60 Extended Study Panel

The Technicon SMA (Sequential Multiple Analyzer) 12/60 System automatically performs 12 biochemical analyses on each serum specimen at the rate of 60 specimens per hour. A serum specimen of approximately 2.0 ml is used for the 12 determinations. Results are reported in concentration units on a moving strip of precalibrated chart paper. The instrument is operated according to the instructions in the *Operation Manual for the Technicon SMA 12/60 AutoAnalyzer.*[50] The SMA 12/60 system is calibrated with a commercial reference serum. Serum calibrator and quality control samples are analyzed with every 10 specimens.

Quality control and calibration of the SMA 12/60.—The calibrator used is "Lederset" (manufactured by the Lederle Company, Division of American Cyanimid, Atlanta, Ga.).

The "standard" is a lyophilized commercially analyzed calibration serum. Serum calibrator is analyzed in duplicate at the beginning of a tray of specimens to calibrate each analytical channel on the SMA 12/60 and is reanalyzed with every 10 samples to check calibration settings.

Quality control materials are:

(a) Scale I—low abnormal and normal, Technicon Instruments Corp., Tarrytown, N.Y.
(b) Scale II—high abnormal and normal, Technicon Instruments Corp.
(c) Ledernorm—normal, Lederle Company, Division of American Cyanimid, Atlanta, Ga.
(d) Ledertrol—high abnormal, Lederle Company.

Scale I and scale II are lyophilized commercial sera reconstituted with 10 ml of accompanying buffers. Ledernorm and Ledertrol are lyophilized commercial sera reconstituted with 5 ml distilled water. At least one of these four quality control samples is analyzed with each 10 specimens and each quality control sample is analyzed at least twice a day.

The following eight analytes were examined on the SMA 12/60 for HANES I:

1. Alkaline Phosphatase

   The SMA 12/60 alkaline phosphatase method is the Morgenstern et al. modification[51] of the Bessey-Lowry-Brock method.[52] The procedure is based on the enzymatic hydrolysis of p-nitrophenyl phosphate at 37° C. After it is incubated at 37° C, the free p-nitrophenyl phosphate is dialyzed into a 2-amino-2-methyl-1-propanol (AMP) buffer stream. Dialysis eliminates the interference of bilirubin and the need for blank correction. The product, p-nitrophenol, is highly colored under alkaline conditions. The absorbance of the alkaline p-nitrophenol is measured in a 15-mm flowcell at 410 nm. Results are reported in International Units per liter at 37° C (IU/l). Repeat limits are below 15 and above 95 IU/l.

   Monthly means for bench quality control pools for alkaline phosphatase with corresponding estimates of imprecision are shown in figure 13.

Estimates of imprecision: Serum alkaline phosphatase (SMA 12/60)

Bench quality control					
Pool number	Dates	Average (IU/l)	Estimated standard deviation (IU/l)	Coefficient of variation (%)	Approximate degrees of freedom[1]
1	Dec. 73 Nov. 75	37.5	2.80	7.50	220
2	Dec. 73 Nov. 75	198.5	7.04	3.50	250
4	Dec. 73 Dec. 74	102.2	4.03	3.90	54
6	Dec. 74 Nov. 75	41.0	2.99	7.30	149
8	Feb. 73 July 73	91.6	3.97	4.30	15
9	Mar. 72 Nov. 73	42.1	4.54	10.80	65
11	Oct. 71 Oct. 73	48.0	6.71	14.00	59
83	Mar. 72 Jan. 73	119.0	6.98	5.90	30

[1]Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

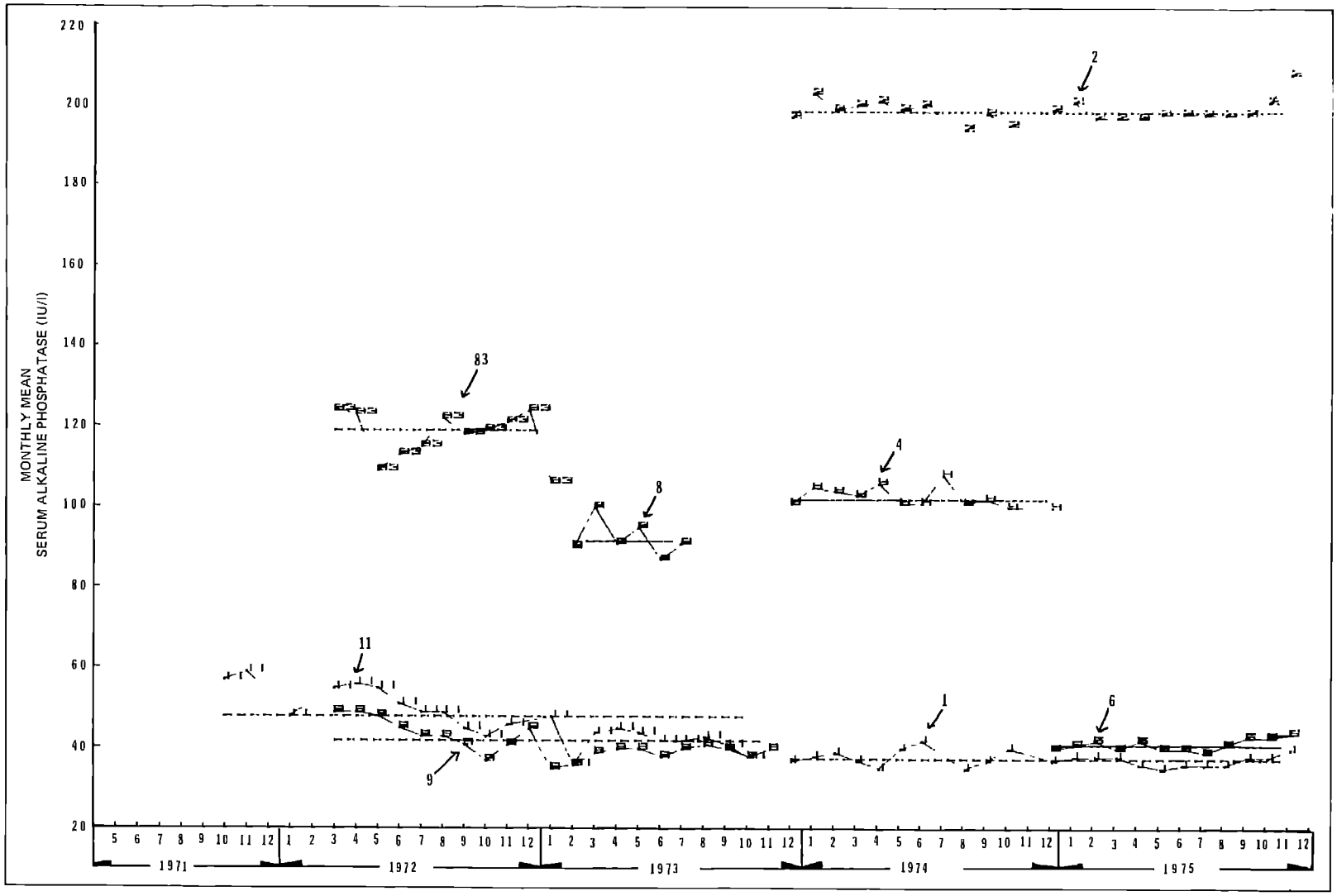

Figure 13. Monthly means for bench quality control pools: Serum alkaline phosphatase (SMA 12/60)

2. Blood Urea Nitrogen (BUN)

The SMA 12/60 BUN procedure is a modification of the method of Marsh et al.[53] In this procedure, a colored product is formed when urea in a relatively weak acid solution reacts with diacetyl-monoxime in the presence of ferric ions. The reaction mixture is heated to 90° C to increase the rate at which color develops. The absorbance of the colored product is measured in a 15-mm flowcell at 520 nm. Results are reported as milligrams per deciliter (mg/dl). Repeat limits are below 5.0 and above 30 mg/dl.

Monthly means for bench quality control pools for BUN with corresponding estimates of imprecision are shown in figure 14.

Estimates of imprecision: Serum blood urea nitrogen (SMA 12/60)

Bench quality control

Pool number	Dates	Average (mg/dl)	Estimated standard deviation (mg/dl)	Coefficient of variation (%)	Approximate degrees of freedom[1]
1	Dec. 73 Nov. 75	15.2	0.64	4.20	213
2	Dec. 73 Nov. 75	46.6	1.19	2.60	263
4	Nov. 73 Dec. 74	89.2	1.83	2.10	92
5	Dec. 74 Nov. 75	8.3	0.47	5.70	150
6	Dec. 74 Nov. 75	12.6	0.61	9.90	147

[1]Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

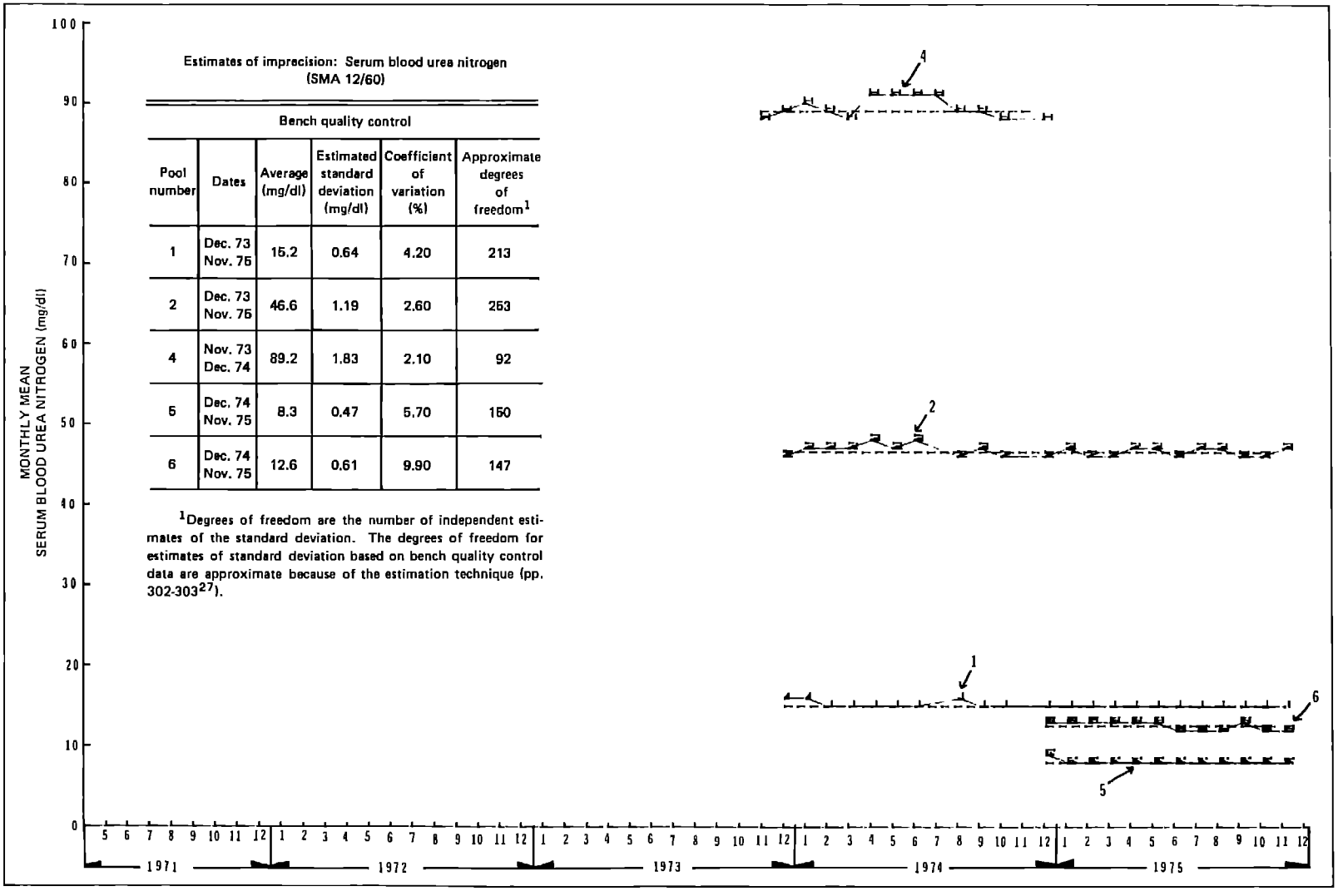

Figure 14. Monthly means for bench quality control pools: Serum blood urea nitrogen (SMA 12/60)

3. Calcium

The SMA 12/60 calcium method is an adaptation of the Gitelman[54] modification of the Kessler and Wolfman method.[55] The diluted serum specimen is mixed with 0.3 mol/l HCl containing 8-hydroxyquinoline. The 0.3 mol/l HCl releases the protein-bound calcium and the 8-hydroxyquinoline combines with magnesium, eliminating its interference in the reaction. The calcium is dialyzed into cresolphthalein complexone containing 8-hydroxyquinoline. A colored complex is formed between calcium and cresolphthalein complexone when diethylamine is added. The absorbance of the colored complex is measured in a 15-mm flowcell at 570 nm. Results are reported as milligrams per deciliter (mg/dl). Repeat limits are below 8 and above 12 mg/dl.

Monthly means for bench quality control pools for calcium with corresponding estimates of imprecision are shown in figure 15.

Estimates of imprecision: Serum calcium (SMA 12/60)

Bench quality control					
Pool number	Dates	Average (mg/dl)	Estimated standard deviation (mg/dl)	Coefficient of variation (%)	Approximate degrees of freedom[1]
1	Dec. 73 Nov. 75	9.12	0.208	2.30	214
2	Dec. 73 Nov. 75	12.64	0.330	2.60	214
4	Nov. 73 Dec. 74	12.15	0.310	2.50	120
5	Dec. 74 Nov. 75	6.10	0.151	2.50	127
8	Feb. 73 July 73	12.00	0.280	2.30	41
11	Oct. 71 Oct. 73	9.58	0.152	1.60	140
83	Jan. 72 Jan. 73	11.70	0.265	2.30	39

[1]Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

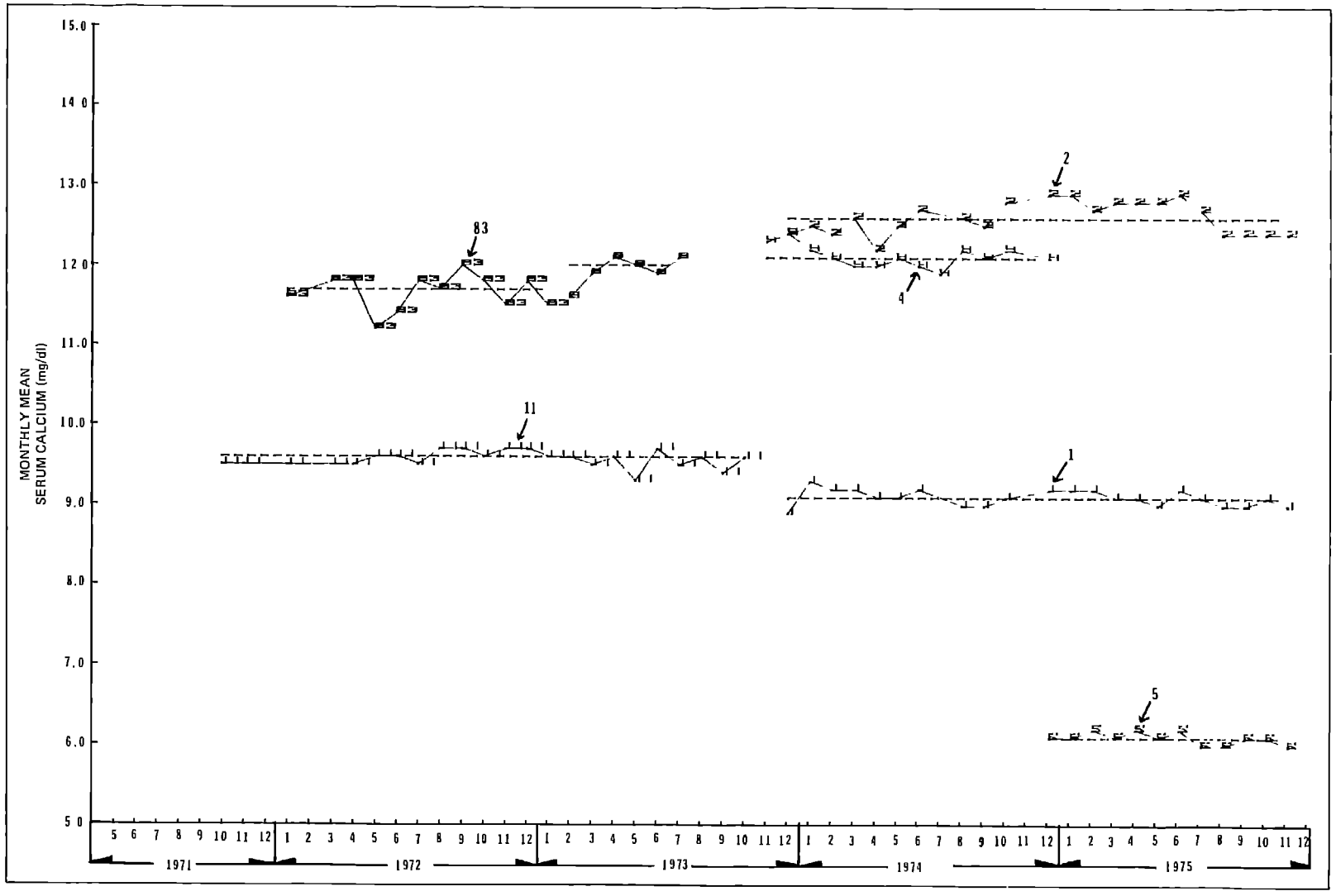

Figure 15. Monthly means for bench quality control pools: Serum calcium (SMA 12/60)

4. Creatinine

The SMA 12/60 creatinine method is the Stevens and Skeggs[56] automation of the alkaline picrate method of Jaffe.[57] The serum specimen is diluted with 1.8% NaCl and is dialyzed into distilled water. The dialysate is mixed with 0.5 mol/l NaOH and then with saturated picric acid. A colored complex between creatinine and alkaline picrate is formed. The absorbance of the colored complex is measured in a 15-mm flowcell at 505 nm. Results are reported as milligrams per deciliter (mg/dl). Repeat limits are below 0.1 and above 2.0 mg/dl.

Monthly means for bench quality control pools for creatinine with corresponding estimates of imprecision are shown in figure 16.

Estimates of imprecision: Serum creatinine (SMA 12/60)

Bench quality control

Pool number	Dates	Average (mg/dl)	Estimated standard deviation (mg/dl)	Coefficient of variation (%)	Approximate degrees of freedom[1]
1	Dec 73 Nov 75	0.95	0.113	11.90	261
2	Dec. 73 Nov. 75	8.38	0.201	2.40	264
4	Nov. 73 Dec. 74	8.12	0.209	2.58	108
5	Dec. 74 Nov. 75	0.82	0.123	14.95	138
6	Dec. 74 Nov. 75	1.17	0.141	12.11	145

[1]Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

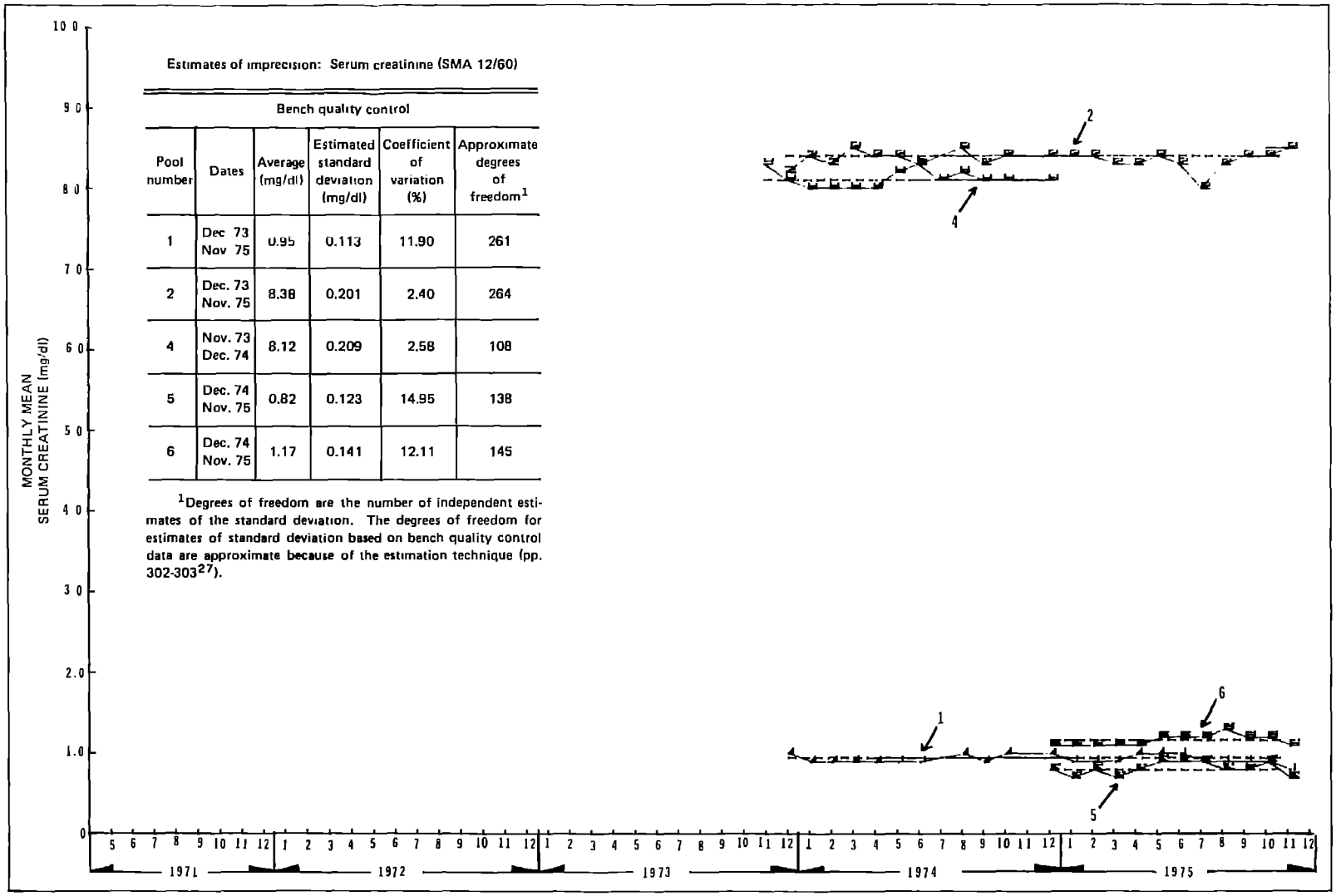

Figure 16. Monthly means for bench quality control pools: Serum creatinine (SMA 12/60)

5. Inorganic Phosphate

The SMA 12/60 inorganic phosphate method is an adaptation of the methods of Hurst[58] and Kraml.[59] Serum is diluted with and then dialyzed against 1% H_2SO_4. The dialysate containing inorganic phosphate is mixed with an acidic solution of ammonium molybdate to form phosphomolybdic acid. This is immediately reduced by stannous chloride-hydrazine to form heteropolymolybdenum blue. The absorbance of this product is measured at 600 nm. Results are reported as milligrams per deciliter (mg/dl). Repeat limits are below 2.0 and above 5.0 mg/dl.

Monthly means for bench quality control pools for inorganic phosphate with corresponding estimates of imprecision are shown in figure 17.

Estimates of imprecision: Serum inorganic phosphate (SMA 12/60)

Bench quality control					
Pool number	Dates	Average (mg/dl)	Estimated standard deviation (mg/dl)	Coefficient of variation (%)	Approximate degrees of freedom[1]
1	Dec. 73 Nov. 75	3.58	0.106	3.00	206
2	Dec. 73 Nov. 75	8.29	0.345	4.20	116
4	Nov. 73 Dec. 74	6.49	0.227	3.50	48
8	Feb. 73 July 73	6.18	0.213	3.50	14
9	Mar. 72 Nov. 73	3.09	0.134	4.30	65
11	Oct. 71 Oct. 73	3.40	0.199	5.90	173
83	Oct. 72 Jan. 73	6.27	0.216	3.50	26
6	Dec. 74 Nov. 75	6.50	0.241	3.70	142

[1]Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

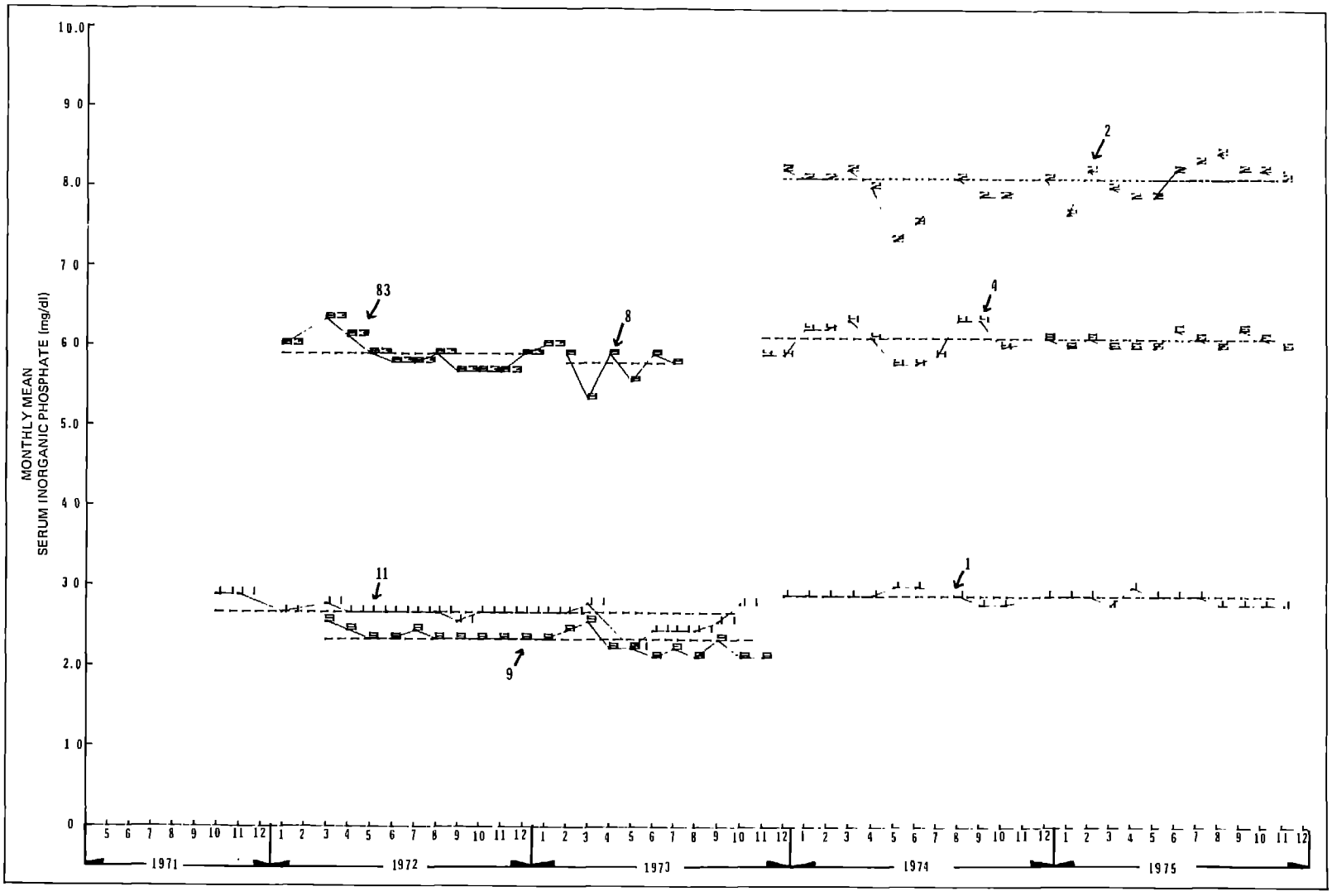

Figure 17. Monthly means for bench quality control pools: Serum inorganic phosphate (SMA 12/60)

6. Uric Acid

The SMA 12/60 uric acid method is the Musser and Ortigoza automation[60] of the Sobrinho-Simões method.[61] Serum is diluted with 0.9% NaCl and is then dialyzed into a sodium tungstate-hydroxylamine solution. Phosphotungstic acid is added to the dialysate. Uric acid reduces phosphotungstic acid to tungsten blue. The absorbance of the tungsten blue is measured in a 15-mm flowcell at 600 nm. Results are reported as milligrams per deciliter (mg/dl). Repeat limits are below 2 and above 8 mg/dl.

Monthly means for bench quality control pools for uric acid with corresponding estimates of imprecision are shown in figure 18.

Estimates of imprecision: Serum uric acid (SMA 12/60)

Bench quality control					
Pool number	Dates	Average (mg/dl)	Estimated standard deviation (mg/dl)	Coefficient of variation (%)	Approximate degrees of freedom[1]
1	Dec 73 Nov. 75	5.25	0.128	2.40	206
2	Dec. 73 Nov 75	11 05	0.338	3.10	137
4	Nov. 73 Dec 74	9 32	0.184	2.00	100
7	Feb. 73 July 73	4.09	0.056	1.40	43
8	Feb 73 July 73	8.71	0.108	1.20	40
9	Mar. 72 Nov. 73	7 18	0.127	1.80	100
11	Oct. 71 Oct 73	6.16	0.169	2.80	120
73	Jan. 72 Jan 73	4.07	0.073	1.80	32
83	Jan. 72 Jan. 73	8.74	0 179	2.00	29

[1]Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

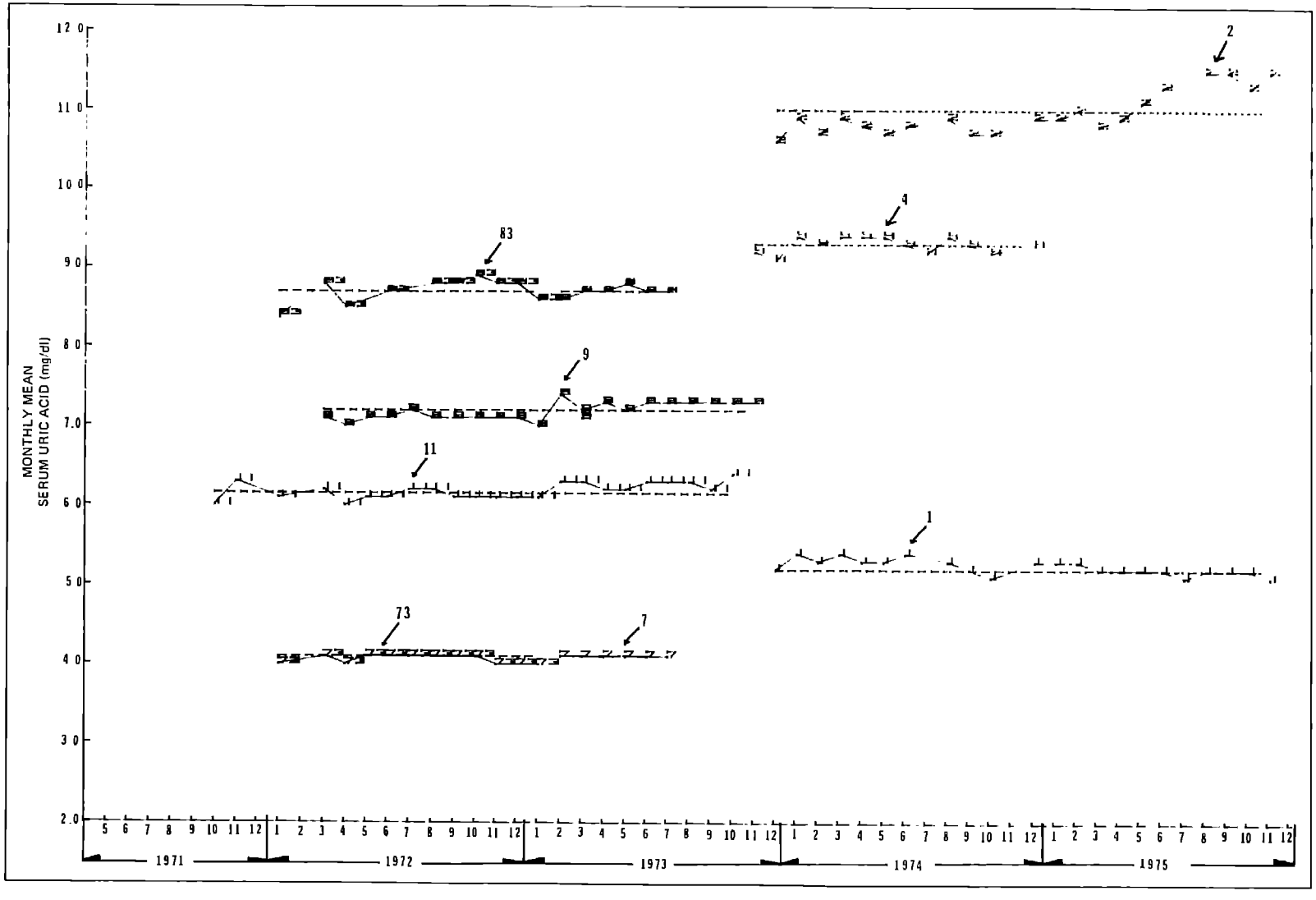

Figure 18. Monthly means for bench quality control pools: Serum uric acid (SMA 12/60)

7. Total Bilirubin

The SMA 12/60 method for total bilirubin is based on the procedure of Jendrassik and Grof[62] as automated by Gambino and Schreiber.[63] Diluted serum is mixed with diazotized sulfanilic acid in the presence of caffeine-sodium benzoate reagent. Bilirubin reacts with diazotized sulfanilic acid to form a colored product, azobilirubin. The azobilirubin solution is made alkaline, and the absorbance of the alkaline azobilirubin is measured in a 15-mm flowcell at 600 nm. A serum blank for each specimen is automatically subtracted by differential colorimetry. Results are reported as milligrams per deciliter (mg/dl). Repeat limits are below 0.2 and above 2.0 mg/dl.

Monthly means for bench quality control pools for total bilirubin with corresponding estimates of imprecision are shown in figure 19.

Estimates of imprecision: Serum total bilirubin (SMA 12/60)

Bench quality control					
Pool number	Dates	Average (mg/dl)	Estimated standard deviation (mg/dl)	Coefficient of variation (%)	Approximate degrees of freedom[1]
1	Dec. 72 Nov. 75	0.49	0.096	19.50	164
2	Dec. 73 Nov. 75	6.23	0.267	4.30	233
4	Nov. 73 Dec. 74	7.37	0.321	4.30	83
8	Feb. 73 July 73	6.55	0.232	3.50	37
9	Mar. 72 Nov. 73	1.22	0.124	10.10	89
11	Oct. 71 Nov. 73	0.29	0.080	27.10	109
83	Jan. 72 Jan. 73	6.62	0.722	10.90	31

[1]Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

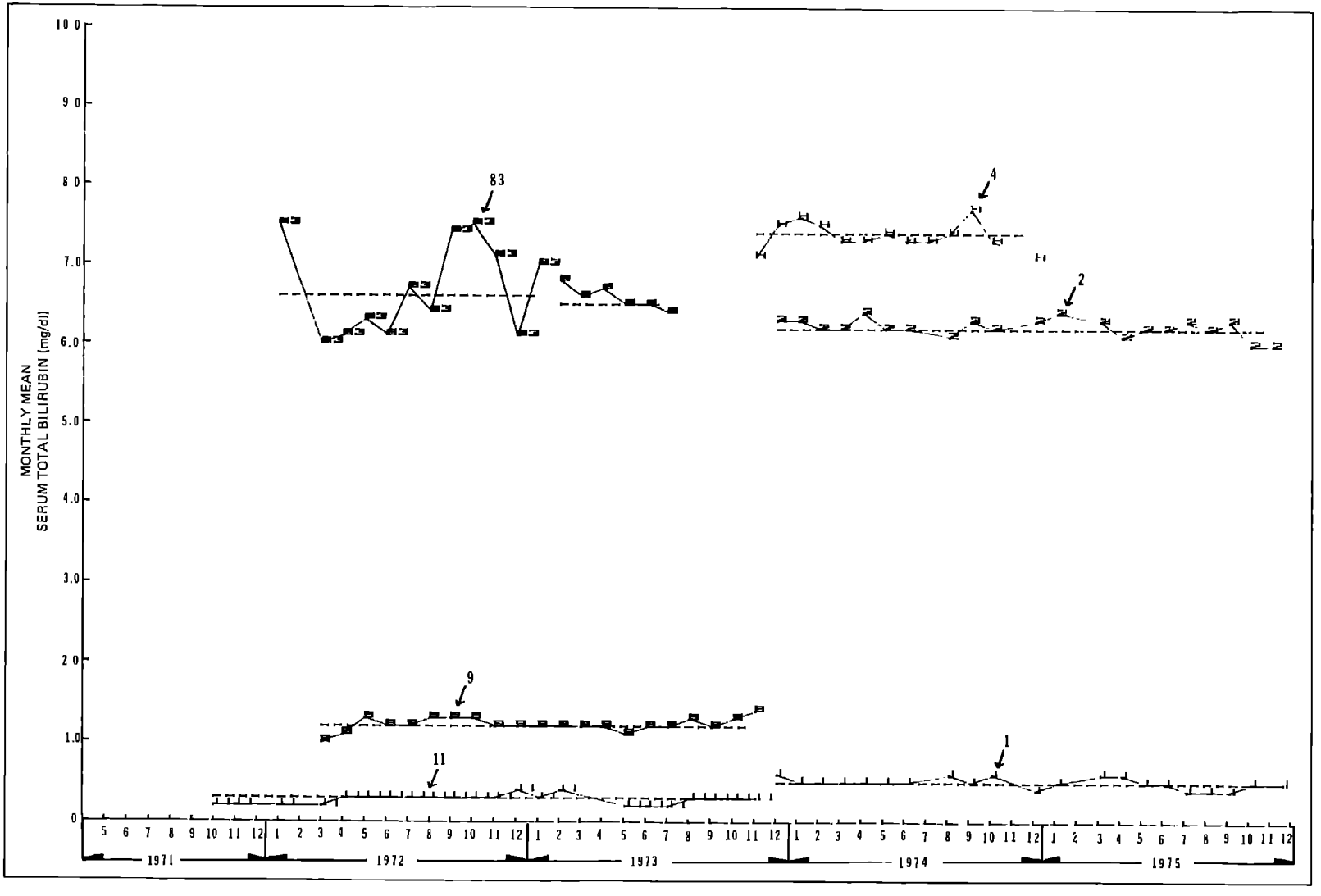

Figure 19. Monthly means for bench quality control pools: Serum total bilirubin (SMA 12/60)

D. Individual Urinary Laboratory Procedures and Quality Control Summaries

1. Iodine

Urinary iodine is determined with a modification of the Benotti et al.[64] adaptation of the Benotti and Benotti method.[65] Iodine is determined indirectly by its catalytic effect on the cerium (IV)-arsenic (III) [Ce(IV)-As(III)] redox reaction. Iodine is quantitated by measuring the disappearance of the yellow Ce(IV) color at 420 nm after a reaction time of 20 minutes at 37° C.

Procedure.—0.1 ml of urine specimen, quality control sample, or standard is diluted with 0.4 ml distilled water in a digestion tube. Then 1.0 ml chloric acid containing 1 mg/ml potassium chromate redox indicator is added to each digestion tube. The samples are digested in an aluminum heating block at 120-130° C until red chromium trioxide crystals precipitate (approximately 2 hours). The digested samples are cooled and diluted with 2.0 ml arsenious acid solution.

A 1.6-ml aliquot of the diluted digestion mixture is mixed with 1.6 ml H_2SO_4 and 2.0 ml water, and then reacted with 1.4 ml ceric ammonium sulfate solution. The final reaction mixture is incubated at 37° C for 20 minutes. The percent transmittance of the unreacted yellow Ce(IV) in the final reaction mixture is measured in a 10-mm flowcell with a Beckman DB-GT Spectrophotometer at 420 nm.

Iodine standards are prepared from analytical grade potassium iodide. The following concentrations of potassium iodide standards are digested and analyzed in duplicate with every 80-120 urine specimens: 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 μg/ml. Urinary iodine concentrations are calculated from the slope and *y*-intercept of the standard curve. Two quality control samples are digested and analyzed with every 20 urine specimens. Urinary iodine is reported as micrograms of iodine per milliliter of urine (μg/ml). Repeat limits are below 0.1 and above 1.0 μg/ml.

The following are noted as modifications of the original method of Benotti et al.:[64] (a) An aluminum heating block is used for the digestion; (b) specimens with concentrations less than 0.1 μg/ml are redigested using 0.5 ml undiluted urine, and then reanalyzed; (c) 1.0 ml chloric acid is used in the digestion; and (d) the rate of analysis is 30 specimens per hour.

Initially, iodine was isolated for analysis by an automated dialysis procedure. Because of the technical problems with the automated dialysis procedure, the digestion procedure of Benotti et al.[64] was eventually adopted.

Monthly means for bench quality control pools for urinary iodine with corresponding estimates of imprecision are shown in figure 20.

Estimates of imprecision: Urinary iodine

Bench quality control					
Pool number	Dates	Average (μg/dl)	Estimated standard deviation (μg/dl)	Coefficient of variation (%)	Approximate degrees of freedom[1]
1	June 71 Sept. 71	0.515	0.0440	8.60	38
2	Aug. 71 Sept. 72	0.680	0.0232	3.40	123
3	Oct. 71 Jan. 72	0.302	0.0142	4.70	43
7	May 72 July 72	0.312	0.0344	11.00	15
8	June 72 Oct. 72	0.433	0.0284	6.60	72
46	Jan. 72 June 72	0.263	0.0258	9.80	51
9	Nov. 72 June 74	0.279	0.0155	5.60	228
11	Aug. 71 Mar. 74	0.191	0.0087	4.60	154
12	Mar. 74 June 74	0.289	0.0153	5.30	81
13	June 74 July 74	0.847	0.0167	2.00	22
14	June 74 July 74	0.158	0.0067	4.30	41
15	Nov. 72 Dec. 72	0.358	0.0127	3.60	19

[1]Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

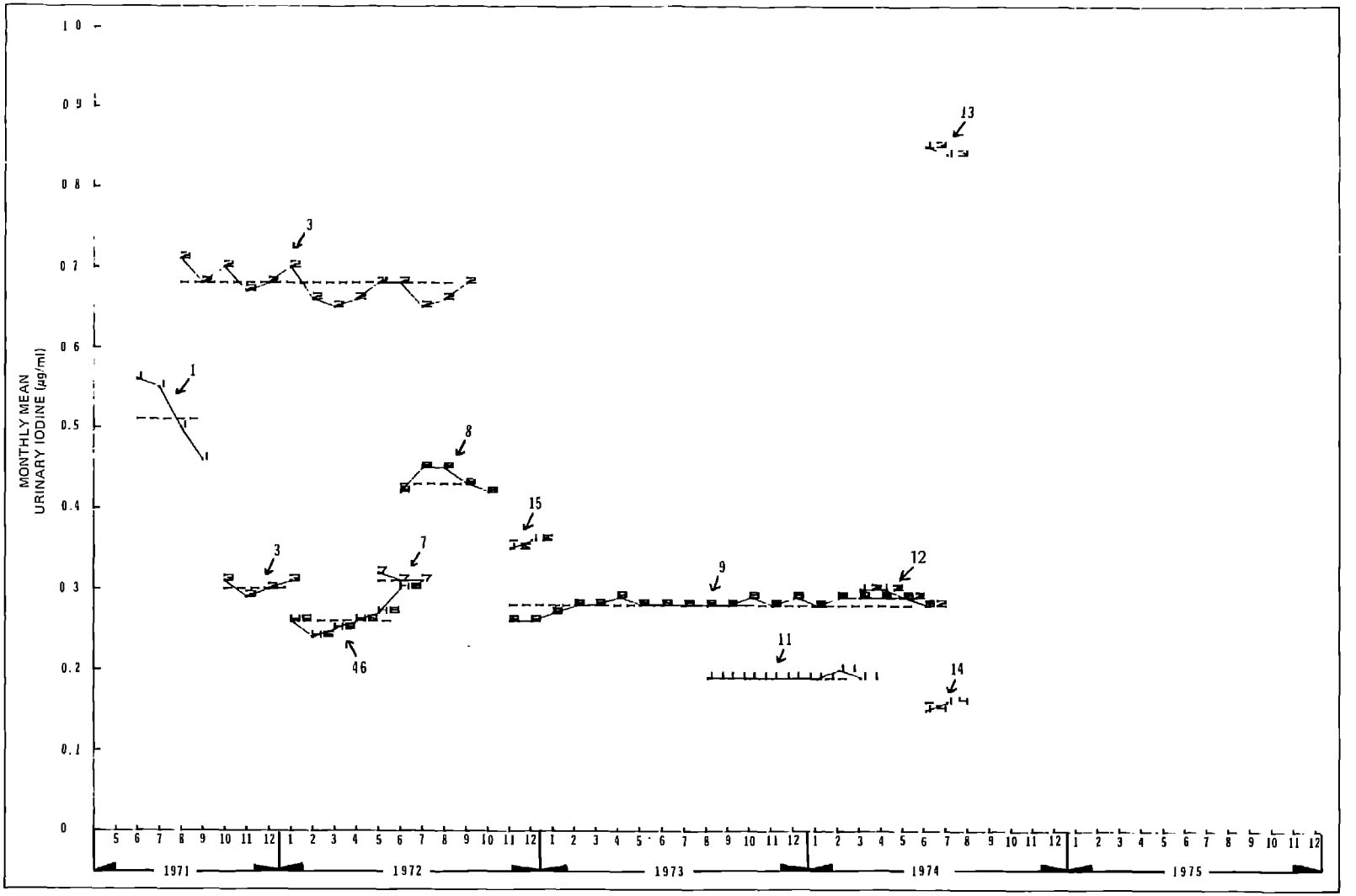

Figure 20. Monthly means for bench quality control pools: Urinary iodine

2. Riboflavin

Urinary riboflavin is quantitated in urine by measuring its fluorescence after it is extracted into an *n*-butanol-pyridine mixture with Pelletier's automated modification[66] of the manual method of Slater and Morell.[67]

Procedure.—0.23 ml of urine specimen, standard, or quality control sample is diluted with 1.6 ml acetic acid-hydrochloric acid solution. The diluted sample is reacted with 1% potassium permanganate to oxidize interferences. The excess potassium permanganate is reduced with a dilute hydrogen peroxide solution. Riboflavin is then extracted into 7.3 ml *n*-butanol-pyridine solution. The fluorescence of this organic phase is measured with a Farrand Ratio Fluorometer at an excitation wavelength of 460 mn and an emission wavelength of 525 nm in a 3-mm flowcell.

To correct for the contribution of nonoxidizable fluorescent interferences, the riboflavin in the urine specimen is decomposed by the irradiation of 2 ml of each urine specimen with a 100-w light source for 16 hours in a rotating rack. The irradiated specimen is analyzed as a urine blank.

Riboflavin standards are prepared from analytical grade riboflavin. The following concentrations of riboflavin standards are analyzed in duplicate with every 20 urine blanks: 0.25, 0.50, 0.75, and 1.0 μg/ml. The concentrations of fluorescent material in urine specimens and urine blanks are calculated from the slope and *y*-intercept of their respective standard curves. The concentration of riboflavin in the urine specimen is computed by subtracting the concentration of the urine blank from the concentration of total fluorescent material. Quality control samples are analyzed with every 20 specimens. Urinary riboflavin is reported as micrograms of riboflavin per milliliter of urine (μg/ml). Repeat limits are below 0.025 and above 1.0 μg/ml.

The following are noted as modifications of the original method of Pelletier:[67] (a) Irradiation time of urine blanks is standardized at 16 hours with a rotating rack around a fixed 100-w light source; (b) to minimize carryover, a wash cup of water is placed between each sample; and (c) because urine samples are received frozen and are thawed on the day of analysis, irradiated blanks are analyzed on the following day.

Monthly means for bench quality control pools for urinary riboflavin with corresponding estimates of imprecision are shown in figure 21.

Estimates of imprecision: Urinary riboflavin

Bench quality control						Quality surveillance							
						Pooled material					Sample resubmission[1]		
Pool number	Dates	Average (μg/ml)	Estimated standard deviation (μg/ml)	Coefficient of variation (%)	Approximate degrees of freedom[2]	Dates	Average (μg/ml)	Estimated standard deviation (μg/ml)	Coefficient of variation (%)	Degrees of freedom[2]	Concentration (μg/ml)	Estimated standard deviation (μg/ml)	Coefficient of variation (%)
2	Aug. 71 July 72	0.22	.019	8.5	182								
7	May 72 Aug. 72	0.42	.014	3.3	48								
3	Sept. 71 Nov. 71	0.47	.012	2.5	308								
1	May 71 Aug. 71	0.55	.048	8.8	26								
4	Jan. 72 May 72	1.51	.033	2.2	93								
5	Jan. 72 May 72	1.57	.048	3.1	82								
8	June 72 Jan. 73	2.70	.052	1.9	340								
0	Feb. 73 Jan. 74	0.26	.009	3.5	885	Apr. 74 June 74	0.31	0.051	16.4	13	0.25	0.050	20.0
6	Mar. 73 July 74	0.35	014	4.0	329	Nov 73 Feb. 74	0.32	0.049	15.6	33	0.35	0.068	19.5
→	July 73 July 74	0.50	022	4.4	409						0.50	0.095	19.0
9	Sept. 72 Jan. 74	0.59	.019	3.3	805						0.60	0.113	18.9
						Apr. 74 June 74	0.73	0.059	8.0	10	0.75	0.140	18.7

[1]Estimates of standard deviation based on 143 sample resubmissions between October 1973 and June 1974; 1 pair (0.7%) was omitted from calculations.

[2]Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

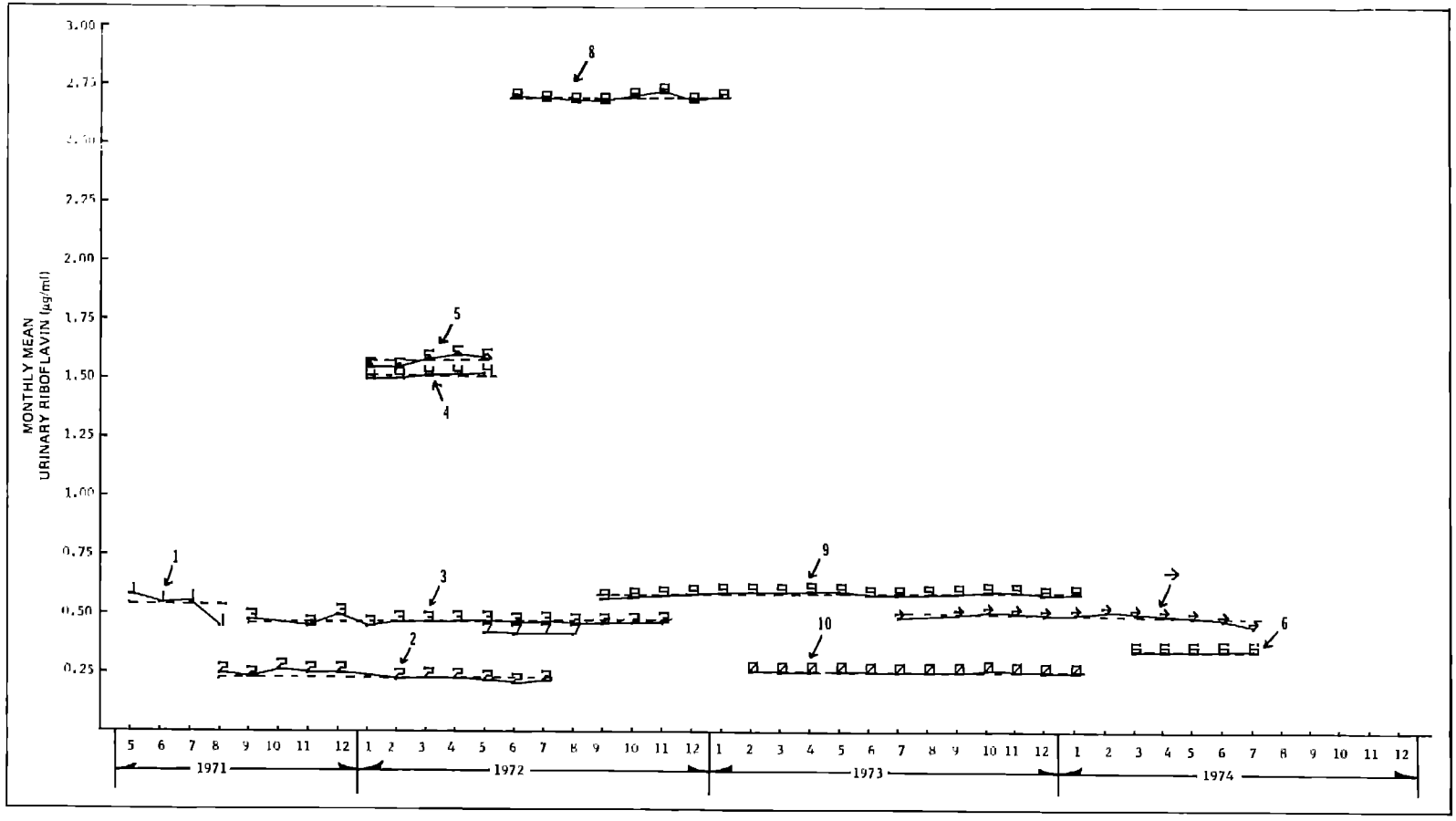

Figure 21. Monthly means for bench quality control pools: Urinary riboflavin

3. Creatinine

Urinary creatinine is quantitated by measuring the intensity of the red complex formed in the reaction between creatinine and alkaline picrate at 505 nm by a modification of the Stevens and Skeggs[56] automation of the alkaline picrate method of Jaffe.[57]

Procedure.—A urine specimen, standard, or quality control sample is diluted 1:500 with water, and a 0.015-ml aliquot is mixed with 1.6 ml alkaline picrate solution. The mixture is incubated for 15 minutes at 30° C, and the absorbance of the resulting red complex is then measured in a 15-mm flowcell with a colorimeter at 505 nm.

Creatinine standards are prepared from analytical grade creatinine. The following concentrations of creatinine standards are analyzed in duplicate with every 60 specimens: 50, 100, 150, 200, 250, and 300 mg/dl. Urinary creatinine concentrations are calculated from the slope and y-intercept of the standard curve. Quality control samples are analyzed with each set of standards and with every 20 specimens. Urinary creatinine is reported in milligrams of creatinine per deciliter of urine (mg/dl). Repeat limits are below 10 and above 300 mg/dl.

The following is noted as a modification of the automated method of Stevens and Skeggs.[56] An unpublished comparison study conducted at CDC resulted in comparable values being obtained for urine specimens analyzed for creatinine with the Stevens and Skeggs[56] method with and without dialysis. Similar observations have been reported in the literature by Henry[68] and Narayanan and Appleton.[69] The method of Stevens and Skeggs[56] without dialysis was adopted.

Monthly means for bench quality control pools for urinary creatinine with corresponding estimates of imprecision are shown in figure 22.

Estimates of imprecision: Urinary creatinine

Bench quality control						Quality surveillance							
						Pooled material					Sample resubmission[1]		
Pool number	Dates	Average (mg/dl)	Estimated standard deviation (mg/dl)	Coefficient of variation (%)	Approximate degrees of freedom[2]	Dates	Average (mg/dl)	Estimated standard deviation (mg/dl)	Coefficient of variation (%)	Degrees of freedom[2]	Concentration (mg/dl)	Estimated standard deviation (mg/dl)	Coefficient of variation (%)
7	May 72 July 72	117	4.6	3.9	21								
6	Jan. 72 May 72	119	3.2	2.7	50								
5	Jan. 72 May 72	120	2.2	1.9	86								
8	June 72 Dec. 72	132	2.9	2.2	194								
0	May 71 Jan. 72	150	7.6	5.1	94								
4	June 74 July 74	63	2.4	4.1	16						63	3.6	5.8
1	Aug. 73 Mar. 74	91	3.3	3.7	51	Apr. 74 June 74	88	2.6	2.9	10	91	4.7	5.2
9	Sept. 72 May 74	110	3.7	3.4	226	Apr. 74 June 74	112	4.3	3.8	11	110	5.5	5.0
2	Mar. 74 June 74	125	5.9	4.7	28						125	6.0	4.8
						Nov. 73 Feb. 74	144	6.8	4.7	33	144	6.8	4.7
3	June 74 July 74	182	4.0	2.2	22						182	8.2	4.5

[1] *Estimates of standard deviation are* based on 145 sample resubmissions between October 1973 and June 1974; 3 pairs (2.1%) were omitted from calculations.

[2] Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp. 302-303[27]).

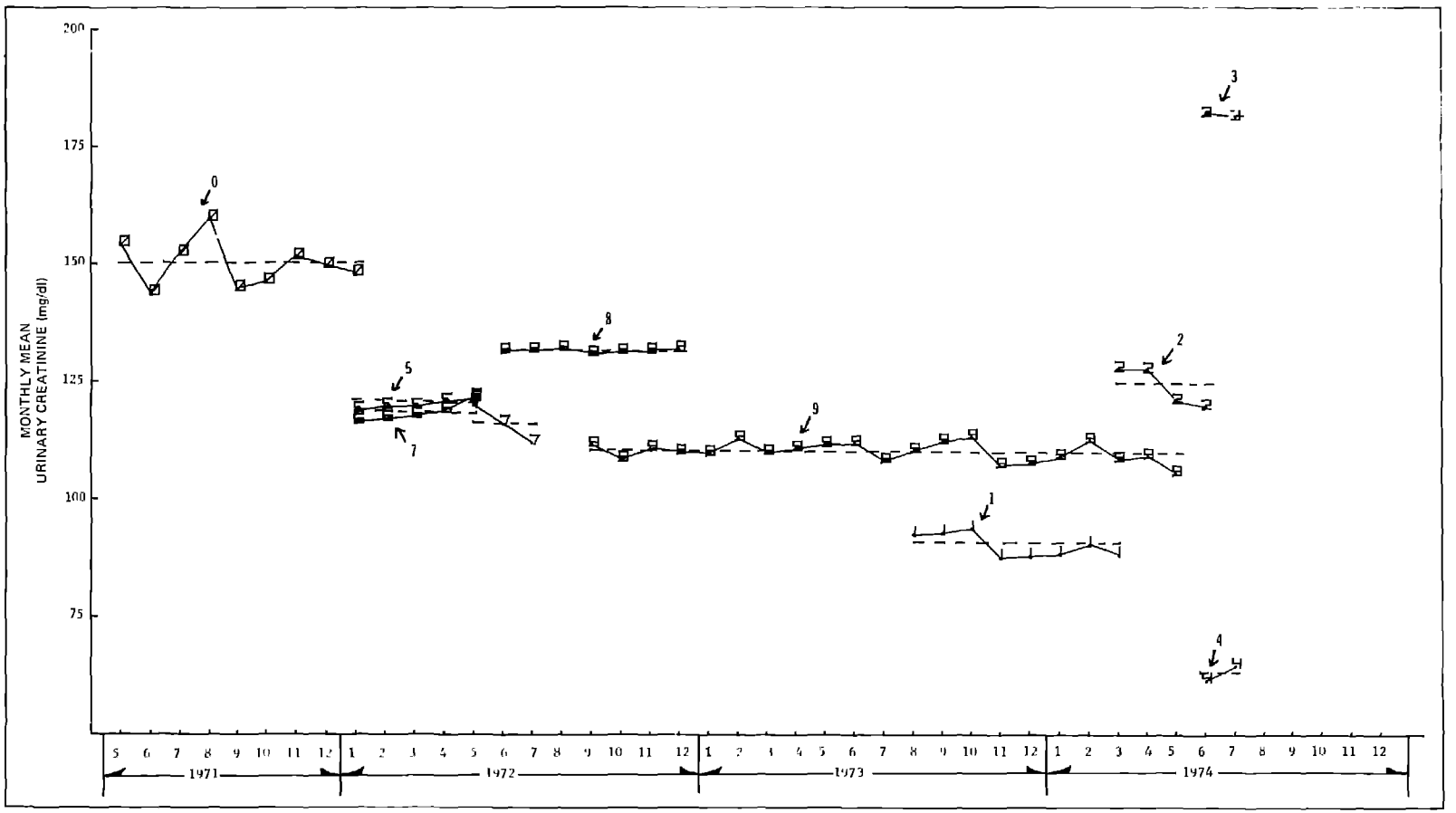

Figure 22. Monthly means for bench quality control pools: Urinary creatinine

4. Thiamine

Urinary thiamine is determined by the automated method of Pelletier and Madere,[70] which is based on the method described in the *Laboratory Manual for Use in Nutrition Surveys.*[71] Thiamine in urine is quantitated by oxidizing thiamine to thiochrome with alkaline potassium ferricyanide. The fluorescence of the thiochrome is measured after thiochrome is extracted into isobutanol.

Procedure.—A 3-ml aliquot of standard, quality control sample, or urine specimen is chromatographed through Decalso ion-exchange columns to remove interfering substances. The thiamine is eluted from the column with 10 ml of 25% KCl in 0.1 mol/l HCl at 94-99° C. 0.16 ml of the thiamine-KCl eluate is diluted with 2.0 ml of 0.1 mol/l HCl, which is then mixed with 0.6 ml potassium ferricyanide in KOH to oxidize the thiamine to thiochrome. The thiochrome is then extracted into 3.9 ml isobutanol. The fluorescence of this organic phase is measured in a 3-mm flowcell with a Farrand Ratio Fluorometer at an excitation wavelength of 360 nm and an emission wavelength of 415 nm.

A blank correction is made for fluorescent interferences in the thiamine-KCl eluate by analyzing a second aliquot of the thiamine-KCl eluate omitting potassium ferricyanide from the reaction mixture. The corrected fluorescence is the difference between the fluorescence of the blank and the total fluorescence. The corrected fluorescence is used in calculating urine thiamine concentration. Thiamine standards are prepared from USP thiamine hydrochloride. The following concentrations of thiamine standards are chromatographed and analyzed in duplicate with every 20 urine specimens: 0.1, 0.3, 0.5, and 0.7 μg/ml. Urinary thiamine concentrations are calculated from the slope and y-intercept of the standard curve with the corrected fluorescence of the thiochrome. Two quality control samples are chromatographed and analyzed with every 20 urine specimens. Urinary thiamine is reported as micrograms of thiamine per milliliter of urine (μg/ml). Repeat limits are below 0.03 and above 0.8 μg/ml.

The following are noted as modifications of the original method of Pelletier and Madere:[72] (a) To minimize carryover between samples, the rate of analysis was decreased from 30 specimens per hour to 20 per hour; (b) initially only 0.1 and 0.3 μg/ml thiamine standards were analyzed with each 20 specimens. Later, 0.5 and 0.7 μg/ml thiamine standards were included in the analysis to verify the linearity over the working range.

Monthly means for bench quality control pools for urinary thiamine with corresponding estimates of imprecision are shown in figure 23.

Estimates of imprecision: Urinary thiamine

Bench quality control						Quality surveillance							
						Pooled material					Sample resubmission[1]		
Pool number	Dates	Average (μg/ml)	Estimated standard deviation (μg/ml)	Coefficient of variation (%)	Approximate degrees of freedom[2]	Dates	Average (μg/ml)	Estimated standard deviation (μg/ml)	Coefficient of variation (%)	Degrees of freedom[2]	Concentration (μg/ml)	Estimated standard deviation (μg/ml)	Coefficient of variation (%)
2	Aug. 71 Dec. 71	0.168	0.014	8.2	124								
7	May 72 Aug. 72	0.226	0.014	6.3	63								
3	Aug. 71 Feb. 72	0.258	0.026	10 2	38								
1	May 71 Aug. 71	0 358	0.049	13.6	49								
8	June 72 Jan. 73	0.464	0.019	4.1	169								
6	Jan. 72 May 72	0.658	0.021	3.2	141								
5	Jan. 72 Apr. 72	0 673	0.021	3.1	98								
→	July 73 July 74	0.199	0.016	8 1	237	Apr. 74 June 74	0 223	0.021	9.4	13	0.20	0.027	13.3
9	Dec. 72 Mar 74	0.248	0 018	7.2	326	Nov. 73 Feb. 74	0.225	0.031	13.9	32	0.25	0.033	13.3
4	Mar 74 July 74	0.416	0.024	5.8	106						0.42	0.056	13.3
0	Feb. 73 Feb 74	0 471	0 026	5.5	252	Apr 74 June 74	0.471	0.025	5.4	10	0.47	0.063	13.3

[1]Estimates of standard deviation based on 181 sample resubmissions between April 1973 and December 1973; 4 pairs (2.2%) were omitted from calculations

[2]Degrees of freedom are the number of independent estimates of the standard deviation. The degrees of freedom for estimates of standard deviation based on bench quality control data are approximate because of the estimation technique (pp 302-303[27]).

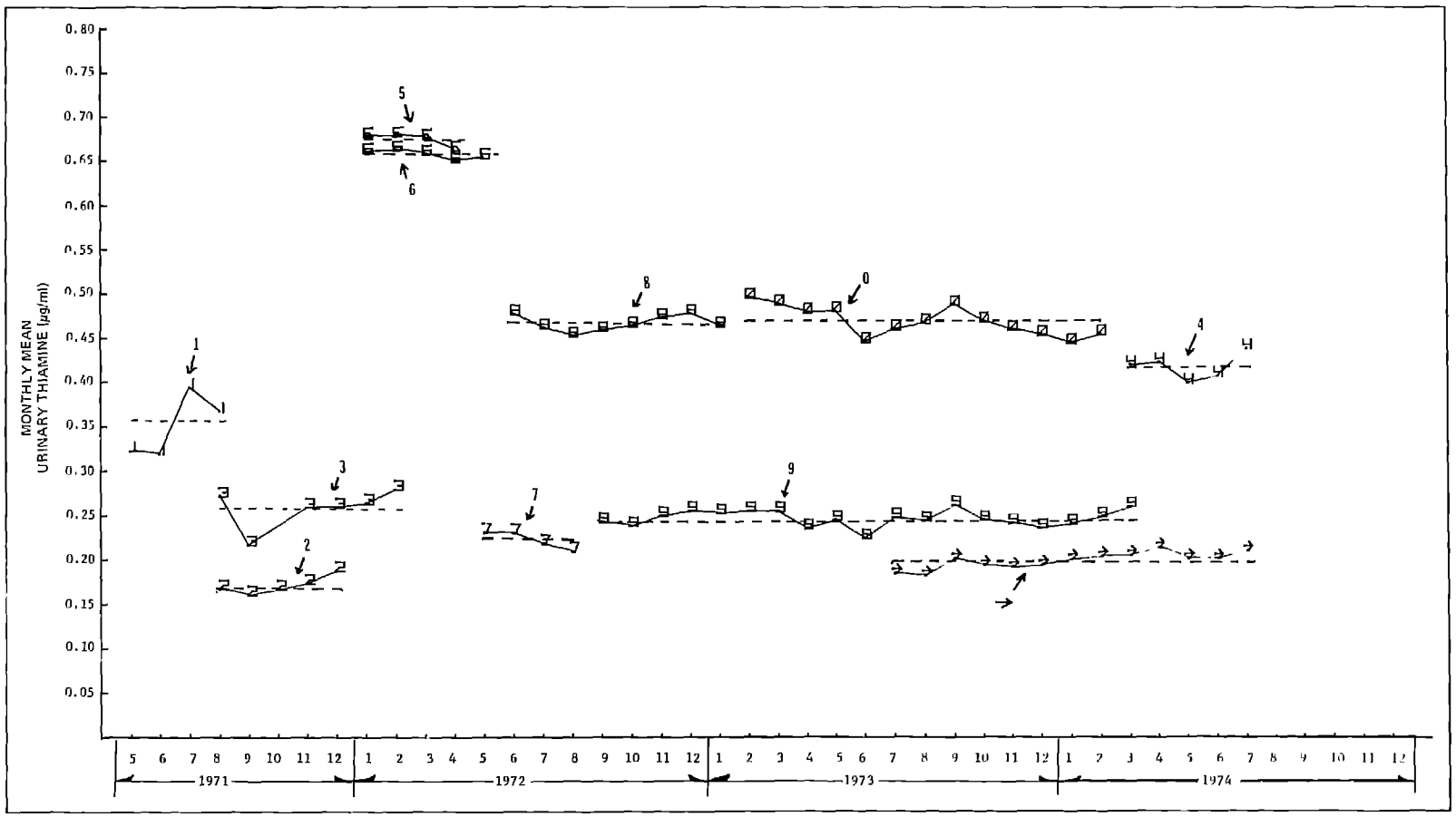

Figure 23. Monthly means for bench quality control pools: Urinary thiamine

VI. STATISTICAL QUALITY CONTROL SYSTEM

It has long been recognized that some variation is inevitable in any repetitive process. For example, Grant states: "Measured quality of manufactured product is always subject to a certain amount of variation as a result of change. Some stable 'system of chance causes' is inherent in any particular scheme of production and inspection. Variation within this stable pattern is inevitable. The reasons for variation outside this stable pattern may be discovered and corrected. . . ."[72]

Although Grant was referring specifically to manufactured products, the statement is equally applicable to determinations made on analytical samples by clinical laboratories. Even when the same analyst repeats a test on a sample with no apparent change in conditions, a slightly different result is often obtained. Greater variations typically occur with changes in factors such as the analyst, instrumentation, reagents, or primary standards.

The phrases "stable pattern" or "in control" are used in this section to denote that with respect to time either (a) no change occurs in the mean (average) value of measurements made on samples known to have the same concentration of a constituent or (b) variation about this mean value remains stable. The phrases "an unstable pattern has developed" or "out of control" are used to denote that either (a) a change has occurred in the mean value or (b) there has been some *increase* in the variation about this mean.

The quality control system described here provides a means of quantifying the variation in a laboratory process when a stable pattern is present and, on the basis of these values, of defining criteria for declaring a particular analytical run outside the stable pattern. Specific objectives are to:

(1) Describe the data necessary to implement and maintain statistical control charts.
(2) Present a brief discussion concerning the use and interpretation of control charts, and
(3) Give detailed instructions for constructing and maintaining control charts.

A. Quality Control Experiment

Although there are many experimental designs available, an experiment that has proved effective in the Clinical Chemistry Division Laboratories, CDC, is described.

1. Control Pool Material

Various types of material can be used as "quality control samples (pools)." Laboratories at CDC normally pool sera (human or animal) or urine to obtain desired con-

centrations of the constituents being examined, making every effort to satisfy the following:

(a) Normal levels of substances known or suspected to interfere with the determination of interest in analytical samples must be present in the control material.
(b) The control material must remain stable over a known period of time. In most cases it is necessary to freeze or lyophilize the control samples.
(c) The total supply must be homogeneous.

If available, two control pools should be used, one with the normal concentration of the constituent being measured and a second with a clinically abnormal concentration of the constituent.

2. Experiment

In each analytical run, the concentrations of all constituents of interest must be determined in k ($k = 1, 2, 3, \ldots$) vials from each pool concurrently with those in unknown samples. The following information should be recorded for each constituent and control pool (see table 1).

(a) Date of analysis
(b) Observed levels of constituent
(c) Run mean ($\bar{X}$) – average of the k observed levels
(d) Within-run range (R) – difference between the highest and lowest observed levels
(e) Sum of the k observed levels

B. Quality Control Charts and Control Criteria

Before giving details for constructing control charts (section VI.C), the language of statistical quality control charts will be introduced and criteria for declaring an unstable pattern will be discussed. The following terms will be used in this and subsequent paragraphs without formal definitions:

- Control level
- Lower control limit (LCL)
- Upper control limit (UCL)
- Within-run variation
- Among-runs variation

The visual record (the control chart) is a plot of each $\bar{X}$ and R value (y axis) as it is obtained versus the number of the run (x axis) in which the determination was made (see figure 24). A separate control chart is made for each control pool. The scale of $\bar{X}$ and R plots should allow for the likely spread of results.

Before the control charts can be completed, measurements on each control pool must be collected for *at least 20 runs of normal operation. During this period, it is extremely important to obtain a true picture of amounts and types of variation present in the process.*

Every effort should be made to ensure that equipment is properly calibrated and methodology is correctly applied. On the other hand, operations should be routine and results should be rejected only if their discrepancies can be directly attributed to such factors as errors in recording or some malfunction in apparatus. To reiterate, a measurement obtained during this initial stage should not be rejected merely because it is not typical of the rest of the data. Sometimes such "outliers" arise from an unusual combination of circumstances which cannot be avoided and which, therefore, should be at least initially included in the control charts being constructed. This subject will be discussed further in section VI.E.

The most important factor in obtaining a true picture of variation in determinations may be the rounding pattern followed by the analyst. Rounding off control sample results may conceal variation to such an extent that it is impossible to construct accurate control charts. To quote Youden,

> Somewhere, many years ago, the idea was implanted in many students that it was completely wrong to retain the uncertain last place or two in the data. "Think how people will be misled and believe that these last place figures really mean something," so the students were told. Well, they do mean something; in fact, they mean everything when it comes to estimating standard deviations (the measure of variation to be used later) or making control charts. Competent, knowledgeable workers are in no danger of being misled by retention of these figures. Never weaken the data by throwing away the very figures indispensable for studying the variation in the results. . . .[73]

We, therefore, recommend that the analyst retain as many figures as possible of control sample results, even if the last figure in a result is an educated guess.

After 20 runs of control sample results have been collected, control limits are calculated according to instructions given in section VI.C.[c] The limits are indicated on control charts by horizontal lines that intersect the vertical scale at the calculated value. Criteria for declaring that a pattern in some future run is unstable are determined by the following statistical interpretation of the control limits:

- $\bar{X}$ chart
  If a stable pattern persists in the long run, approximately 99%[d] (the selected control level) of future run means will be found between the upper and lower control limits; about 1% of these means will fall either below the lower limit or above the upper limit. If an unstable pattern develops, more than 1% of future run means will fall outside the limits.

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[c]Upper and lower control limits are calculated for the $\bar{X}$ chart, whereas only an upper limit is calculated for the R chart; the logic will be apparent later.

[d]It should be noted that this percent is strictly accurate when certain distributional properties of errors are satisfied and estimates of variation are very reliable; otherwise, this percent is approximate. Our past experience with data obtained from serum-constituent analyses indicates that these statistical conditions are reasonably satisfied and that the stated control level is a good approximation of the true control level.

- R chart
  If a stable pattern persists in the long run, approximately 99% of future run range values will fall below the upper control limit, and about 1% will be found above it. If an unstable pattern develops, more than 1% of the future run range values will be located above the upper limit.

This frequency (long run) interpretation is interesting, but a laboratory must decide after each run whether its determinations demonstrate a stable pattern. Suppose that a particular value of $\overline{X}$ or R falls outside a 99% control limit. A laboratory is then faced with two alternatives: (a) A stable pattern exists but includes a rare event (one which occurs 1 in 100 times in the long run) or (b) an unstable pattern has developed and this event is not rare. Since alternative (a) depends on the occurrence of such a rare event, condition (b) should be checked for.

It is suggested that the control levels of 95% and 99% and a center line be used on each chart (see figure 24). The system should then be declared "out of control" (unstable) if any of the following events occur:

- $\overline{X}$ chart
  (a) A single $\overline{X}$ value falls above the upper 99% limit or below the lower 99% limit.
  (b) Two successive $\overline{X}$ values fall either both above the upper 95% limit or both below the lower 95% limit.
  (c) Eight $\overline{X}$ values in succession fall either all above the center line or all below the center line.

- R chart
  (a) A single R value falls above the upper 99% limit.
  (b) Two successive R values fall above the 95% upper limit.
  (c) Eight R values in succession fall above the center line.

If the system should be declared out of control, the following remedial action should be taken:

(a) Check for recording errors of control sample results.
(b) If no recording errors are found, check and calibrate instruments before performing further analyses on analytical samples.
(c) Reanalyze patient samples performed during the out-of-control run.

C. Calculation of Control Limits

Before control limits can be computed, it is necessary to quantify the amount of variation in the initial set of data (data from first 20 runs). The means of measuring variation suggested here differ somewhat from those commonly associated with control charts. (See pp. 81-83,[72] for example.) This difference is dictated by the fact that in the clinical laboratory, it is not unusual to observe more variation among determinations on identical samples made in different runs than among determinations made on identical samples in the same run. Of course, this phenomenon is not unique to serum constituent determinations, but may be more prevalent than in most manufacturing processes and must be considered when control charts are constructed.

In statistical terminology, the total variation in our process contains two components, (1) a within-run component of variation and (2) an among-runs component of variation. The particular quantity to be used here to measure variation is "sample[e] variance,[f]" roughly[g] defined for a set of measurements subject to only one component of variation as the average of the squared differences, with the differences being taken from the average of the measurements. With two components of variation, and thus two components of variance, the calculations required are slightly more complicated but easily carried out with an electronic calculator.

Because our objective is to construct both R control charts (run range) and $\overline{X}$ control charts (run mean), it is necessary to estimate expected variation in each of these statistics. The range is subject only to the within-run component of variance, because the among-runs component is in effect canceled out by taking the difference between two measurements made in the same run. The variance of a run mean is subject to a particular combination of both components of variance.[h] Fortunately, the within-run component of variance needed for the R chart and the particular combination of components (within a constant) needed for the $\overline{X}$ chart can both be obtained through a statistical technique called "analysis of variance."

An understanding of the concept of variance components affords a better understanding of the purposes of $\overline{X}$ and R charts. Since R is subject only to within-run variation, an out-of-control point is a good indication of an increase in the within-run variability. Incidentally, only upper control limits are necessary for the R chart, because a decrease in within-run variability is desirable and not a reason to halt the process. An out-of-control point on the $\overline{X}$ chart is a good indication that (1) a change has occurred in the reported mean value, (2) the among-runs variability has increased, (3) the within-run variability has increased, or (4) some combination of these has occurred.

The steps necessary for constructing control limits are now given. The data used (table 1) and the resultant charts (figure 24) to illustrate the steps are actual results from control samples analyzed by the Clinical Chemistry Division, CDC. The following notation will be used:

(1) k will denote the number of measurements per run.
(2) n will denote the number of runs involved in collecting the initial data set.
(3) X_{ij} will denote the jth measurement in the ith run (X_{43} is the 3d measurement in the 4th run).
(4) S_i will denote the sum of the k measurements made in the ith run.
(5) R_i will denote the range of the k measurements made in the ith run.
(6) $\overline{X}_i$ will denote the average of the k measurements made in the ith run.

---

[e]Statisticians make a careful distinction between an estimate of a quantity based on an experimental sample and the value that one would obtain if the number of measurements was infinite.

[f]The more commonly used quantity, "standard deviation," is simply the square root of the variance. Final calculations will be based on standard deviations.

[g]The word "roughly" is used because the sum of squared differences is actually divided by what statisticians call "degrees of freedom," which in the case described here is one less than the number of measurements.

[h]A complete understanding of this idea requires some knowledge of statistical models. The reader is referred to any basic statistical methods text, such as the one by Ostle, p. 237.[27]

Table 1. Glucose control data

Date of analysis	Run number	Observed level		Average $\overline{X}_i$	Range R_i	Sum S_i
		X_{i_1}	X_{i_2}			
6/14/71	1	131.3	130.7	131.00	0.6	262.0
6/18/71	2	134.0	134.0	134.00	0.0	258.0
6/19/71	3	139.4	134.0	136.70	5.4	273.4
6/22/71	4	137.5	133.0	135.25	4.5	270.5
6/23/71	5	135.2	133.0	134.10	2.2	268.2
6/22/71	6	131.6	133.7	132.65	2.1	265.3
6/28/71	7	135.9	134.8	135.35	1.1	270.7
6/29/71	8	136.4	134.1	135.25	2.3	270.5
6/30/71	9	132.4	134.1	133.25	1.7	266.5
7/14/71	10	133.6	134.7	134.15	1.1	268.3
7/15/71	11	134.7	135.8	135.25	1.1	270.5
7/16/71	12	132.4	134.7	133.55	2.3	267.1
7/26/71	13	133.6	134.7	134.15	1.1	268.3
7/27/71	14	135.0	133.2	134.10	1.8	268.2
7/28/71	15	132.6	133.8	133.20	1.2	266.4
10/4/71	16	135.8	133.8	134.80	2.0	269.6
11/9/71	17	138.2	135.7	136.95	2.5	273.9
11/10/71	18	136.9	138.8	137.85	1.9	275.7
11/23/71	19	137.9	136.7	137.30	1.2	274.6
11/29/71	20	136.0	134.7	135.35	1.3	270.7
1/4/72	21	132.2	134.1	133.15	1.9	266.3
1/10/72	22	132.4	133.1	132.75	0.7	265.5
1/17/72	23	134.7	134.0	134.35	0.7	268.7
1/26/72	24	134.3	135.0	134.65	0.7	269.3
2/2/72	25	134.2	132.2	133.20	2.0	266.4
2/23/72	26	134.0	134.7	134.35	0.7	268.7
3/3/72	27	132.7	134.7	133.70	2.0	267.4
3/6/72	28	136.7	137.4	137.05	0.7	274.1
3/14/72	29	135.0	135.0	135.00	0.0	270.0
3/23/72	30	133.3	135.3	134.20	2.0	268.6
3/27/72	31	131.4	133.4	132.40	2.0	264.8
4/4/72	32	135.8	135.8	135.80	0.0	271.6
4/10/72	33	134.0	132.0	133.00	2.0	266.0
4/24/72	34	135.7	132.8	134.25	2.9	268.5
5/1/72	35	132.5	133.2	132.85	0.7	265.7

Exact k values must be computed for each run. If an obvious error should occur in the analysis of a control sample, the result should be ignored and another sample should be substituted in the analytical run. If for some reason a value is missing in a run, the entire run must be deleted from the calculations. The more measurements included in a run, the greater is the chance that an unstable pattern will be discovered immediately.

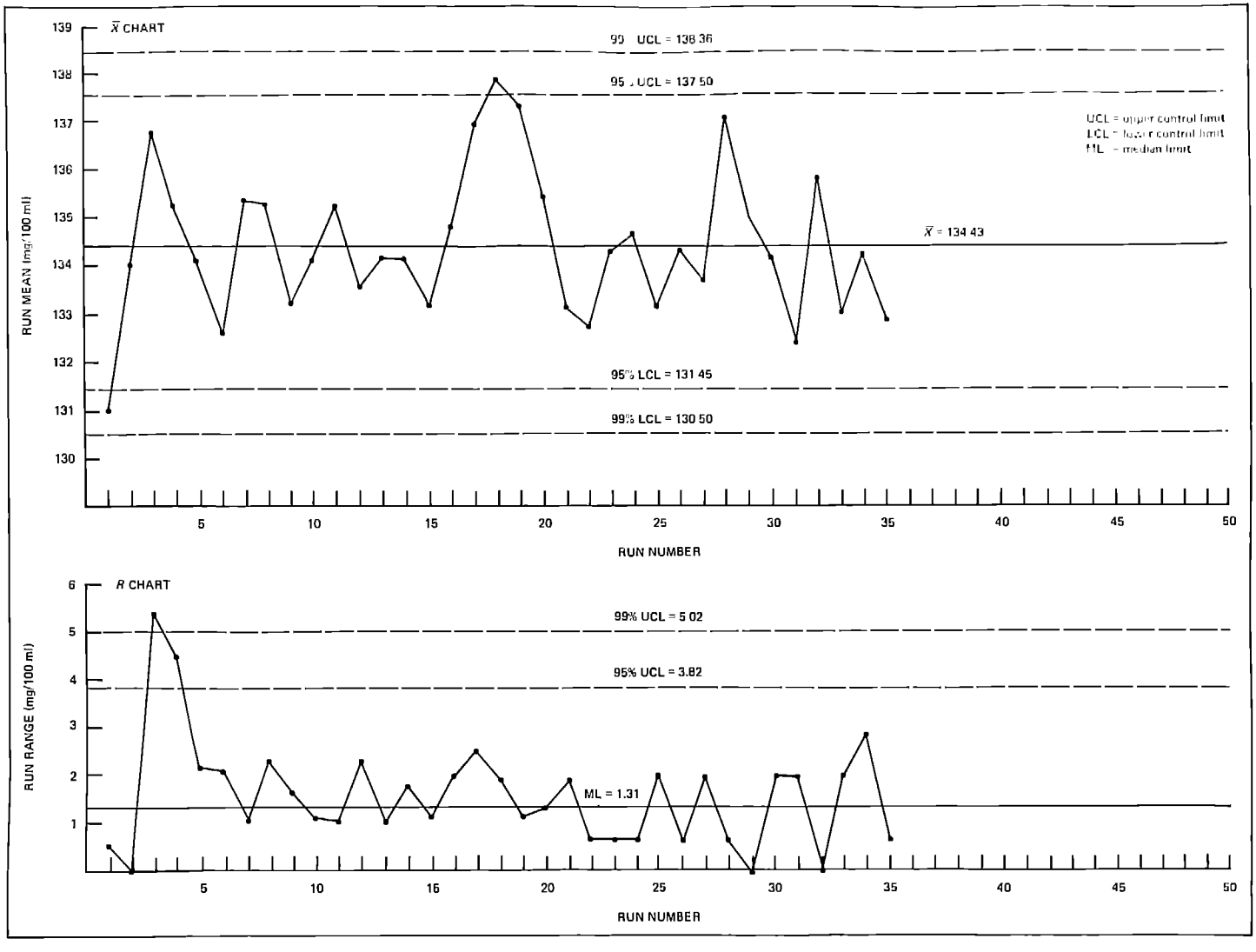

Figure 24. Quality control charts for glucose control data

D. Calculations

1. Computing Steps With Data Given

STEP 1. Compute the total sum of squares (TSS).

$$\text{TSS} = \text{sum of squares of individual values} - \frac{(\text{sum of all values})^2}{\text{total number of values}}$$

$$= X_{11}^2 + \ldots + X_{1k}^2 + X_{21}^2 + \ldots + X_{2k}^2 + \ldots + X_{n1}^2 + \ldots + X_{nk}^2$$

$$- \frac{(X_{11} + \ldots + X_{1k} + \ldots + X_{n1} + \ldots X_{nk})^2}{nk} = (131.3)^2 + (130.7)^2$$

$$+ \ldots + (132.5)^2 + (133.2)^2 - \frac{(131.7 + 130.7 + \ldots + 135.7 + 133.2)^2}{(35)(2)}$$

$$= 224.582857$$

STEP 2. Compute the among-runs sum of squares (ARSS).

$$\text{ARSS} = \frac{(\text{total for first run})^2 + \ldots + (\text{total for last run})^2}{k} - \frac{(\text{sum of all values})^2}{\text{total number of values}}$$

$$= \frac{S_1^2 + S_2^2 + \ldots + S_n^2}{k} - \frac{(S_1 + S_2 + \ldots + S_n)^2}{nk}$$

$$= \frac{(262.0)^2 + (263.0)^2 + \ldots + (265.7)^2}{2} - \frac{(262.0 + 268.0 + \ldots + 265.7)^2}{(35)(2)}$$

$$= 157.602857$$

STEP 3. Compute the within-run sum of squares (WRSS).

$$\text{WRSS} = \text{TSS-ARSS} = 224.582857 - 157.602857 = 56.980000$$

STEP 4. Compute the degrees of freedom (DF).

(a) DF for TSS = total number of values $- 1 = nk - 1 = 70 - 1 = 69$

(b) DF for ARSS = number of runs $- 1 = n - 1 = 35 - 1 = 34$

(c) DF for WRSS = DF for TSS $-$ DF for ARSS $= (nk - 1) - (n - 1) = n(k - 1) = 35$

STEP 5. Compute the mean squares.

(a) Total mean square (TMS) $= \frac{\text{TSS}}{\text{DF for TSS}} = \frac{\text{TSS}}{nk - 1} = \frac{224.582857}{69} = 3.254824$

(b) Among-runs mean square (ARMS) $= \dfrac{\text{ARSS}}{\text{DF for ARSS}} = \dfrac{\text{ARSS}}{n-1}$

$$= \frac{157.602857}{34} = 4.635378$$

(c) Within-run mean square (WRMS) $= \dfrac{\text{WRSS}}{\text{DF for WRSS}} = \dfrac{\text{WRSS}}{n(k-1)}$

$$= \frac{66.98000}{35} = 1.913714$$

STEP 6. Construct an analysis of variance (AOV) table.

Source of variation	DF	Sum of squares	Mean square
Total	$nk - 1 = 69$	TSS = 224.582857	TMS = 3.254824
Among runs	$n - 1 = 34$	ARSS = 157.602857	ARMS = 4.635378
Within runs	$n(k-1) = 35$	WRSS = 66.980000	WRMS = 1.913714

STEP 7.[i] Compute the among-runs (sample) variance (S^2_{AR}) and (sample) standard deviation (S_{AR}).

(a) If ARMS > WRMS,

$$S^2_{AR} = \frac{\text{ARMS} - \text{WRMS}}{k} = \frac{4.635378 - 1.913714}{2} = 1.360832$$

$$S_{AR} = \sqrt{S^2_{AR}} = \sqrt{1.360832} = 1.166547$$

(b) If ARMS $\leq$ WRMS, then $S^2_{AR} = 0$; that is, the observed run-to-run variation did not exceed the variation within a run.

STEP 8. Compute the within-runs (sample) variance (S^2_{WR}) and (sample) standard deviation (S_{WR}).

(a) If ARMS > WRMS,

$$S^2_{WR} = \text{WRMS} = 1.913714$$

$$S_{WR} = \sqrt{1.913714} = 1.383770$$

(b) If ARMS $\leq$ WRMS,

$$S^2_{WR} = \text{TMS}$$

$$S_{WR} = \sqrt{\text{TMS}}$$

[i]This step is not necessary if the sole purpose is to construct control limits.

STEP 9. Compute the run-mean (sample) variance ($S^2_{\bar{X}}$) and (sample) standard deviation ($S_{\bar{X}}$).

(a) If ARMS > WMRS (as is the case for this example),

$$S^2_{\bar{X}} = \frac{\text{ARMS}}{\text{number of values per run}} = \frac{4.635378}{2} = 2.317689$$

$$S_{\bar{X}} = \sqrt{S^2_{\bar{X}}} = \sqrt{2.317689} = 1.522395$$

(b) If ARMS ≤ WRMS,

$$S^2_{\bar{X}} = \frac{\text{TMS}}{\text{number of values per run}} = \frac{\text{TMS}}{k}$$

$$S_{\bar{X}} = \sqrt{S^2_{\bar{X}}}$$

STEP 10. Compute control limits for run-mean ($\bar{X}$) chart.

(a) Overall mean ($\bar{\bar{X}}$)

$$\bar{\bar{X}} = \frac{\text{sum of all values}}{\text{total number of values}} = 134.43$$

(b) Control limits

- 95% limits

$$\text{Lower control limit (LCL)} = \bar{\bar{X}} - (1.96)S_{\bar{X}}$$
$$= 134.43 - (1.96)(1.52)$$
$$= 131.45$$

$$\text{Upper control limit (UCL)} = \bar{\bar{X}} + (1.96)S_{\bar{X}}$$
$$= 134.43 + (1.96)(1.52)$$
$$= 137.50$$

- 99% limits

$$\text{Lower control limit (LCL)} = \bar{\bar{X}} - (2.58)S_{\bar{X}} = 130.50$$

$$\text{Upper control limit (UCL)} = \bar{\bar{X}} + (2.58)S_{\bar{X}} = 138.36$$

Note: The factors 1.96 and 2.58 are such that approximately 95% and 99%, respectively, of future means will fall within the limits if the system remains in control.

STEP 11. Compute control limits for range (R) chart.

(a) 50% limit

$$\text{Median limit (ML)} = C_1 S_{WR} = (0.95)(1.38) = 1.31$$

The factor C_1 is obtained from table 2 and depends on the number of control pool samples analyzed per run (k). The interpretation of this limit (ML) is that if the pattern of within-run variation remains stable, approximately 50% of future range values will fall above ML and 50% will fall below ML.

(b) 95% upper limit

$$\text{Upper control limit (UCL)} = C_2 S_{WR} = (2.77)(1.38) = 3.82$$

(c) 99% upper limit

$$\text{Upper control limit (UCL)} = C_3 S_{WR} = (3.64)(1.38) = 5.02$$

Again the factors are obtained from table 2 and are such that the stated percentage of future range values will be less than the computed limits if the pattern of within-run variation remains stable.

Table 2. Factors for computing range chart control limits

Number of values per run (k)	Multiplier for		
	50% limit (C_1)	95% limit (C_2)	99% limit (C_3)
2	0.95	2.77	3.64
3	1.59	3.31	4.12
4	1.98	3.63	4.40
5	2.26	3.86	4.60
6	2.47	4.03	4.76
7	2.65	4.17	4.88
8	2.79	4.29	4.99
9	2.92	4.39	5.08
10	3.02	4.47	5.16
11	3.12	4.55	5.23
12	3.21	4.62	5.29
13	3.29	4.68	5.35
14	3.36	4.74	5.40
15	3.42	4.80	5.45

2. Computing Steps With Data Missing

If the number of determinations per run is *not* the same for each run of the initial data set (that data used to construct control limits), the following modifications must be made in the notation and steps 1 through 10, in the pages that precede:

Notation:

(a) n will denote the number of runs.

(b) $k_i, i = 1, 2, \ldots, n$, will denote the number of measurements in the ith run.

(c) $N = \sum_{i=1}^{n} k_i$ will denote the total number of measurements.

(d) X_{ij} will denote the jth measurement in the ith run (X_{43} is the 3d measurement in the 4th run.

(e) S_i will denote the sum of the k_i measurements made in the ith run.

STEP 1. Compute the total sum of squares (TSS).

$$\text{TSS} = \text{sum of squares of individual values} - \frac{(\text{sum of all values})^2}{\text{total number of values}}$$

$$\text{TSS} = X_{11}^2 + \ldots + X_{1k_1}^2 + \ldots + X_{n1}^2 + \ldots + X_{nk_n}^2 - \frac{(X_{11} + \ldots + X_{1k_1} + \ldots + X_{n1} + \ldots + X_{nk_n})^2}{N}$$

STEP 2. Compute the among-runs sum of squares (ARSS).

$$\text{ARSS} = \frac{(\text{total for first run})^2}{k_1} + \ldots + \frac{(\text{total for last run})^2}{k_n} - \frac{(\text{sum of all values})^2}{\text{total number of values}}$$

$$= \frac{S_1^2}{k_1} + \frac{S_2^2}{k_2} + \ldots + \frac{S_n^2}{k_n} - \frac{(S_1 + S_2 + \ldots + S_n)^2}{N}$$

STEP 3. Compute the within-run sum of squares (WRSS).

$$\text{WRSS} = \text{TSS} - \text{ARSS}$$

STEP 4. Compute degrees of freedom (DF).

(a) DF for TSS = total number of values $- 1 = N - 1$

(b) DF for ARSS = number of runs $- 1 = n - 1$

(c) DF for WRSS = DF for TSS $-$ DF for ARSS $= (N - 1) - (n - 1) = N - n$

STEP 5. Compute the mean squares.

(a) Total mean square (TMS) $= \dfrac{\text{TSS}}{N-1}$

(b) Among-runs mean square (ARMS) $= \dfrac{\text{ARSS}}{n-1}$

(c) Within-run mean square (WRMS) $= \dfrac{\text{WRSS}}{N-n}$

STEP 6. Construct an analysis of variance (AOV) table.

Source	DF	Sum of squares	Mean square
Total	$N-1$	TSS	TMS
Among runs	$n-1$	ARSS	ARMS
Within runs	$N-n$	WRSS	WRMS

STEP 7a. Compute k_0.

$$k_0 = \frac{\sum_{i=1}^{n} k_i - \sum_{i=1}^{n} k_i^2 \div \sum_{i=1}^{n} k_i}{n-1} = \frac{N - \sum_{i=1}^{n} k_i^2 \div N}{n-1}$$

STEP 7b. Compute the among-runs (sample) variance (S_{AR}^2) and (sample) standard deviation (S_{AR}).

(a) If ARMS $>$ WRMS,

$$S_{AR}^2 = \frac{\text{ARMS} - \text{WRMS}}{k_0}$$

$$S_{AR} = \sqrt{S_{AR}^2}$$

(b) If ARMS $\leqslant$ WRMS, then

$$S_{AR}^2 = 0$$

STEP 8. Compute within-run (sample) variance (S^2_{WR}) and (sample standard deviation (S_{WR}).

(a) If ARMS > WRMS,

$$S^2_{WR} = \text{WRMS}$$

$$S_{WR} = \sqrt{S^2_{WR}}$$

(b) If ARMS $\leqslant$ WRMS,

$$S^2_{WR} = \text{TMS}$$

$$S_{WR} = \sqrt{S^2_{WR}}$$

STEP 9. Compute the (sample) variance (S^2_T) and (sample) standard deviation (S_T) of a single determination.

$$S^2_T = S^2_{WR} + S^2_{AR}$$

$$S_T = \sqrt{S^2_T}$$

STEP 10. Compute the run-mean (sample) variance ($S^2_{\bar{X}}$) and (sample) standard deviation ($S_{\bar{X}}$).

Now, let k^* denote the (constant) number of determinations per run to be made in the future.

(a) If ARMS > WRMS,

$$S^2_{\bar{X}} = \frac{S^2_{WR}}{k^*} + S^2_{AR}$$

$$S_{\bar{X}} = \sqrt{S^2_{\bar{x}}}$$

(b) If ARMS $\leqslant$ WRMS,

$$S^2_{\bar{X}} = \frac{\text{TMS}}{k^*}$$

$$S_{\bar{X}} = \sqrt{S^2_{\bar{x}}}$$

Continue with steps 10 and 11 in section VI.D.1.

STEP 11. Compute control limits for run-mean ($\bar{X}$) chart as in step 10 in section VI.D.1.

E. Revision of Control Limits

After control limits are computed and plotted, it is not unusual for some points in the initial data set to fall outside the 99% limits. These runs should be considered out-of-control. It is suggested that limits be reset once in order to delete these points from the data set; however, a second recalculation is not suggested if additional points fall outside the revised limits. If the original limits were based on only 20 runs, they should be revised after 30 runs, with data from all 30 runs included except for those representing out-of control points. After 30 runs, regular periods of review (such as every month or every 25 runs) should be established.

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VIII. GLOSSARY

Terms are listed in alphabetical order. Undefined terms which are used in their ordinary dictionary senses are assumed to be understood in context.

All terms defined in this glossary (except those marked with an asterisk) are taken from "Provisional Recommendations on the Quality Control in Clinical Chemistry."[74]

*Absorbance: Denoted by A, defined as the negative logarithm (to the base 10) of the transmittance; that is, $A = -\log T = \log (1/T) = \log (100\% T)$.

Accuracy: Agreement between the best estimate of a quantity and its true value. It has no numerical value.

Aliquot: Measured portion of a whole having the same composition. General term referring to any solution, sample, mixture, etc.

*Analysis of variance: The statistical analysis of the variance of a random variable to determine if certain factors associated with the variable contribute to that variance.

Analyte: The component to be measured.

*Best Anemia Classifier: A blood constants calculator for classifying the anemias, distributed by Clay Adams Division of Beckton, Dickinson and Company, Parsippany, N. J. 07054.

Calibration: Process of relating the reading to the quantity required to be measured.

Carryover: The influence of a sample upon a following one.

*Casual urine specimen: A urine specimen obtained at time of examination, not a timed specimen such as a 24-hour collection.

*Combistix: Reagent strip test for the determination of urinary glucose, protein, and pH. (Ames Company, #2867, Division of Miles Laboratories, Elkhart, Ind.)

*Control chart: A graph of a quantitative characteristic of a chemical process, usually determined from small, periodically repeated samples and evaluated with respect to control limits rendered as parallel horizontal lines above and below a line representing the expected or average value of the characteristic.

Control material: Material used for quality control purposes.

Control specimens, bench quality control samples, control solution: Specimen or solution that is analyzed solely for quality control purposes, not for calibration.

Degrees of freedom: The number of free (unrestricted and independent in the sense of random sampling) variables entering into a statistic.

*Extended Study Panel: Additional tests (serum bilirubin, BUN, uric acid, creatinine, calcium, phosphate, SGOT, and alkaline phosphatase) performed on a subsample of HANES I participants ages 25-74 years (stands 1-65) and all participants of HES (stands 66-100).

*Flocculent precipitate: A fluffy or woolly-appearing solid substance that is chemically caused to be separated from a solution.

*Hemolysis: The destruction of red blood cells with the liberation of hemoglobin which diffuses into the serum. Hemolyzed serum would be characterized by a reddish color rather than its normal straw color.

*Icteric: Yellow-hued serum resembling jaundice. The yellow color appears in the serum as the result of certain liver diseases.

Imprecision: Standard deviation or coefficient of variation of the results in a set of replicate measurements. The mean value and number of replicates must be stated, and the design used must be described in such a way that other workers can repeat it. This is particularly important when a specific term is used to denote a particular type of imprecision, such as between-laboratories, within-day, or between-days.

Interference: The effect of a component, which does not by itself produce a reading, on the accuracy of measurement of another component.

*Isoton: Diluent for cell counts distributed by Curtin Matheson Scientific, Inc., Houston, Tex.

*Lipemic: Fat in the blood. A cloudy to opalescent effect caused by high serum cholesterol or fatty acid levels.

Method, analytical: Set of written instructions in which are described the procedure, materials, and equipment that are necessary for the analyst to obtain a result.

*Microdel vial: 3-ml polyethylene vial with stopper and cap.

*Percent transmittance: Denoted by T, the ratio of the radiant power P transmitted by a sample to the radiant power P_0 incident on the sample. Hence, $T = P/P_0$. The percent transmittance is given by $T \times 100$ and may be denoted by % T.

*Plasma vitamin C: Specimens collected for this assay, but data not presented in this report.

Quality control: The study of those errors that are the responsibility of the laboratory, and the procedures used to recognize and minimize them. This study includes all errors arising within the laboratory between the receipt of the specimen and the dispatch of the report. On some occasions, the responsibility of the laboratory may extend to collecting the specimen from the patient and providing a suitable container for it.

Reading: The value indicated on the scale of an instrument or analytical device.

*Repeat limits: Concentration levels for each analyte used to determine when a sample needs to be retested for verification because its concentration is clinically abnormal or is outside the calibration range of the instrument.

Result: Final value obtained for a measured quantity after performing a measuring procedure including all subprocedures and laboratory evaluations.

Run: Usually a set of consecutive assays performed without interruption. The results are usually calculated from the same set of calibration standard readings. However, in cases in which this is not universally applicable, the word "series" should be defined and then used.

Sample: The appropriately representative part of a specimen that is used in the analysis. The term "test sample" should be used when the term might be confused with the statistical term "random sample from a population."

*Serum folate: Specimens collected for this assay, but data for HES only presented in this report.

Specimen: Material available for analysis.

*Stand: Primary sampling unit, a geographical location representing a subsample of the United States population designated by the U.S. Bureau of the Census for the design of the HANES program.

Standard: Material or solution with which the sample is compared in order to determine the concentration. The compound term "calibration standard" should be used as needed to avoid confusion with other technical or colloquial meanings of standard.

U-bag: Plastic bag with adhesive edges designed for urine sample collection from infants and small children, distributed by Scientific Products Company.

Value, true: Term considered to have a self-evident meaning requiring no definition. In practice, true values are approximated by reference method values.

*Wheaton vial: 50-ml amber borosilicate glass vial—catalog number 223785—Wheaton Scientific, Millville, N. J. 08332.

*Zap-Oglobin: Stromatolyzing agent, distributed by Curtin Matheson Scientific, Inc., Houston, Tex.

IX. ABBREVIATIONS

AMP	amino-methyl-propanol buffer used in alkaline phosphatase assay
AOV or ANOVA	analysis of variance
As(III)	arsenic with the +3 oxidation state
BGC	bromcresol green dye, used in serum albumin assay
BUN	blood urea nitrogen
CBC	complete blood count; hematology profile
CDC	Center for Disease Control, Atlanta, Ga. 30333
Ce(IV)	cerium with the +4 oxidation state
DE52	resin used in hemoglobin electrophoresis
DF	degrees of freedom
EDTA	ethylene-diamine-tetra-acetic acid (or ethylenediaminetetraacetic acid)
Fe(II)	iron with the +2 oxidation state
fl	femtoliter, 1.0×10^{-15} liter
G-6-P	glucose-6-phosphate
G-6-PD	glucose-6-phosphate dehydrogenase, red cell enzyme
HANES I	Health and Nutrition Examination Survey I
Hct	hematocrit, packed cell volume
Hb	hemoglobin
HES	Health Examination Survey
HiCN	cyanmethemoglobin
HPO_3	metaphosphoric acid
l	liter, unit of volume
M	mole, unit of concentration
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin content
MCV	mean corpuscular volume
MDH	malate dehydrogenase
μ^3	cubic micron, $(.000001\ M)^3$, measure of red blood cell size
μl	microliter, .000001 liter
ml	milliliter, 1.0×10^{-3} liter
NAD	nicotinamide adenine dinucleotide
NAD^+	oxidized nicotinamide adenine dinucleotide
NADH	reduced nicotinamide adenine dinucleotide
NADP	nicotinamide adenine dinucleotide phosphate
$NADP^+$	oxidized nicotinamide dinucleotide phosphate
NADPH	reduced nicotinamide dinucleotide phosphate
OD	optical density

PGA	pteroylglutamic acid, folic acid
psi	pounds per square inch
RBC	red blood cell count
rev/min	revolutions per minute
SGOT	serum glutamic-oxaloacetic transaminase (aspartate aminotransferase), cardiac/liver enzyme
SMA	Sequential Multiple AutoAnalyzer (Technicon Instruments Corp., Tarrytown, N. Y.)
T_3	triiodothyronine, thyroid hormone
T_4	thyroxine, thyroid hormone
TEB	tris-EDTA-borate buffer
TFA	trifluoroacetic acid
TIBC	total iron-binding capacity
TPN^+	oxidized triphosphopyridine nucleotide
TPNH	reduced triphosphopyridine nucleotide
Tris-HCl buffer	Tris-hydrochloric acid buffer
USP	United States Pharmacopoeia
UV	ultraviolet
veronal buffer	buffer made with sodium barbital and hydrochloric acid
WBC	white blood cell count

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