

## Operating Instructions

# Photoelectric Colorimeter



Clinical Model (Tubes)

Catalog No. T37012-0000  
Catalog No. T37012-0001

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# PRINCIPALS OF OPERATION

This is a Photoelectric Colorimeter using specific light filters of the visible range (380mm-740mm). The scale readings are directly proportional to the concentration in accordance with Beers law.

Two matched photocells of the "blocking layer" type, in a fully compensated and carefully balanced electrical circuit, form the basis for current measurement. The galvanometer is of the suspension wire type.

## SPECIFICATIONS

T37012-0000	Colorimeter	Tube Model 800-3	110V	100W
T37012-0001	Colorimeter	Tube Model 800-3	220V	100W

## OUTSIDE DIMENSIONS

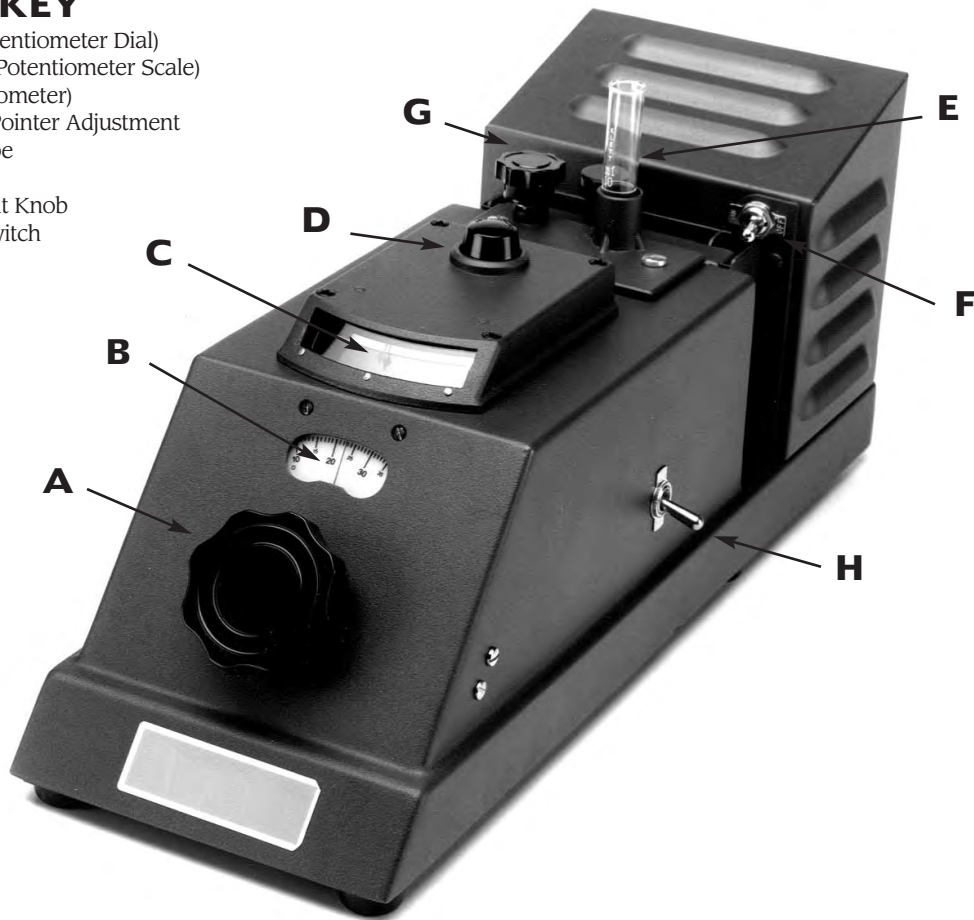
Length:	17.5 in. (44.5cm)	Height:	7.5in (19cm)
Width:	6 in (15cm)	Weight:	12.5 lbs. (5.7 kg)

## SHIPPING SPECIFICATIONS

Size:	12x13x22 in (30.5x3356 cm)	Weight:	21 lbs. (9.5 kg)
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## KEY

- A** Scale Knob (Potentiometer Dial)
- B** Scale Reading (Potentiometer Scale)
- C** Pointer (Galvanometer)
- D** Galvanometer Pointer Adjustment
- E** Colorimeter Tube
- F** Light Switch
- G** Zero Adjustment Knob
- H** Short-Circuit Switch



# CONTROLS

## THE ZERO ADJUSTMENT KNOB

The small knob located at the top of the Colorimeter, to the left of the test tube or solution cell. This knob enables the pointer to be brought to its zero position at the line of the pointer scale when the Colorimeter lamp is on.

## GALVANOMETER ADJUSTMENT KNOB (POINTER)

The zero position for the galvanometer pointer is when the tip of the pointer is exactly on the single line on the pointer scale. The pointer should be in this position when the instrument is not in operation and the lamp is turned off.

All colorimetric measurements are based on the pointer being at its zero position when distilled water or the reagent blank solution is in place and the scale reads 0. The accuracy of the instrument depends to a large extent on how carefully the zero adjustment is made.

**Note:** During the use of the instrument the pointer may occasionally swing to the extreme end of the scale with considerable force. This does no harm provided the pointer is not left for any length of time in that position, but is brought back to the center of the scale as quickly as possible.

## GALVANOMETER SWITCH

To protect the pointer mechanism from damage during shipping, there is a toggle switch (H). This switch should be set to "OFF" **only** when moving or shipping the Colorimeter. For every day use, this switch should be set in the "ON" position, otherwise the pointer will not move when the lamp is turned on.

## LAMP SWITCH

The lamp switch, located on the front of the lamp housing, is designed so that the filters cannot be removed from the Colorimeter without switching the lamp off. This is to minimize the possibility of strong unfiltered light striking the photoelectric cells.

## THE COLORIMETER LAMP

This is a standard 100 watt double filament bulb and operates from the standard electrical AC or DC line. The lamp bulb is not operated at its full rating in order to prolong the life of the lamp.

# KEY ELEMENTS

## LIGHT FILTERS

The light filter should be inserted in the filter holder with the round opening facing forward. To prevent damage to the photocells, the Colorimeter lamp should not be turned on unless there is a filter in place. For best results, insert this filter in the filter holder with the engraved number located in the lower right corner, facing you. Due to variation in the raw glass, filters from different batches may appear to be a different color. This will not affect the transmissivity of the filter.

## CHOICE OF PROPER FILTER

For best results it is important that the proper light filter is used. It has been found that the three most popular filters will cover most ordinary colorimetric requirements. There are also other filters for your needs.

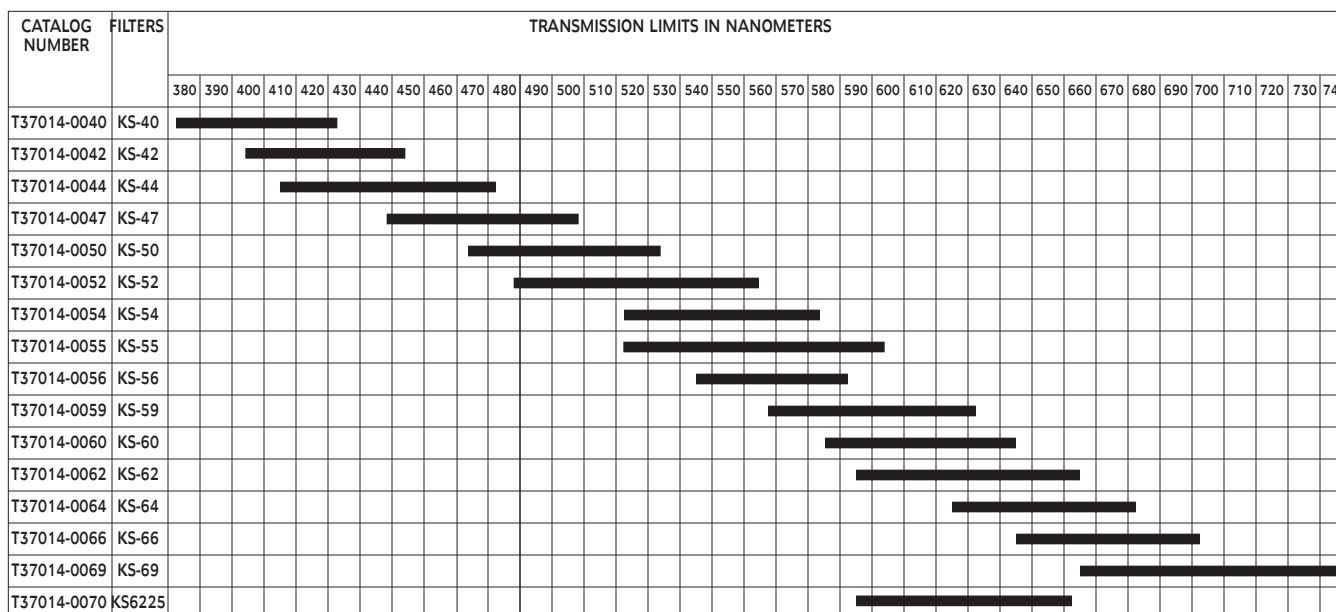
The proper filter is usually specified in the directions for the procedure. Ordinarily the filter selected is the one with a spectral transmission opposite to that of the solution being measured, i.e. the filter which transmits the most light over the range where the solution absorbs the most light. In this way maximum sensitivity is usually obtained. Furthermore, it is frequently possible by use of proper filters to read two colors, both present in the same solution, independently of each other, or to specifically measure one colored compound in the presence of an extraneous color.

When changing from one filter to another, the change in the color of light striking the photoelectric cells may disturb the balance between them temporarily, so the instrument should run for about 5 to 10 minutes with the new filter in place before making measurements.

It is necessary to reset the zero with distilled water when a new filter is in place in the Colorimeter, since the cells in balance for one filter are not necessarily in balance for another.

Filters should not be left in machine with lamp on.

CATALOG NUMBER	FILTER NUMBER	COLOR	APPROXIMATE SPECTRAL RANGE (NANOMETERS)	COLOR OF SOLUTION FOR WHICH FILTER IS USED
T37014-0042	KS-42	BLUE	400-450	RED, ORANGE, YELLOW, GREEN, BLUE, TURBID
T37014-0054	KS-54	GREEN	520-580	RED, YELLOW, PURPLE, ORANGE, BLUE
T37014-0066	KS-66	RED	640-700	BLUE, GREEN, YELLOW



The spectral transmission curve of a typical filter (no. 52 is selected) is illustrated. The spectral specificity of this filter is indicated by the fact that 85% of all the light transmitted has a wavelength between 485 and 550 nanometers.

**TEST TUBES: CAT. NO. T37012-0010 (UNGRADUATED) T37012-0020(GRADUATED)**

The macro test tubes can be heated in boiling water in the event that the colorimetric procedure calls for it. These test tubes may also be used for centrifugation to remove turbidity (as described in TURBIDIMETRIC MEASURES below).

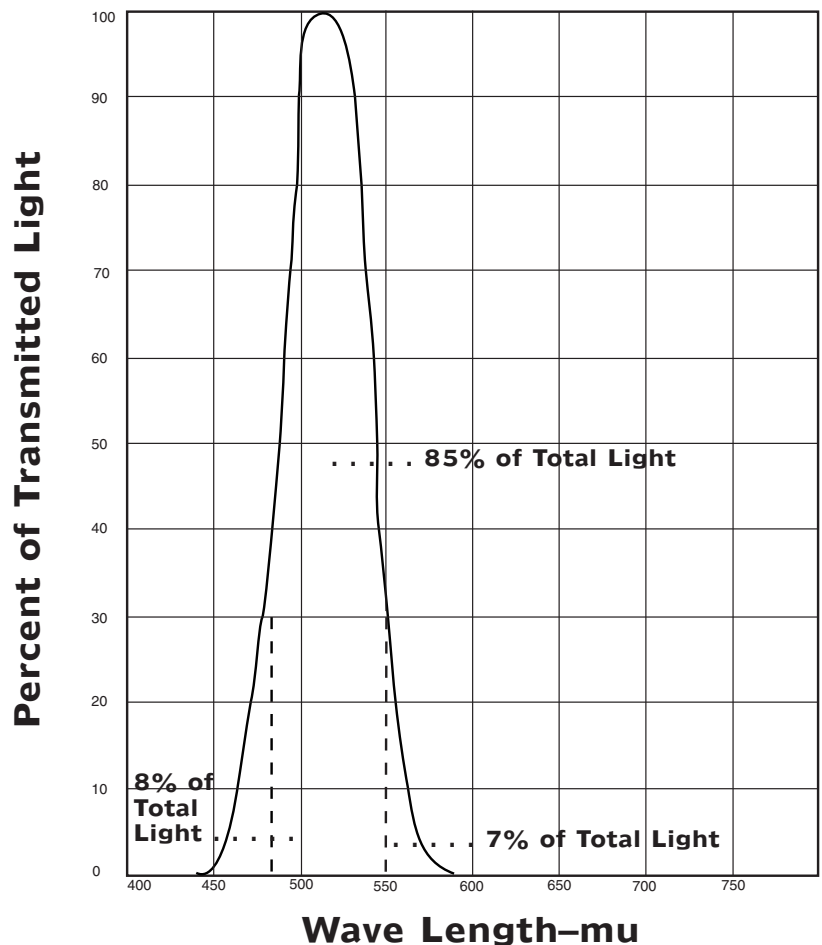
**MICRO TUBES: CAT. NO. 93701-2012 (FLAT BOTTOM, 2.5ML)**

Micro tubes are used for those analytical procedures which require volumes as little as 2.5ml. These tubes are similar to the regular tubes, but have flat bottoms. Care should be taken when adding hot solutions, or heating these tubes in boiling water, since the flat bottoms of the micro tubes are less resistant to heat shock than the rounded bottom macro tubes.

**USE OF COLORIMETER SCALE**

**READING**

The Klett-Summerson Photoelectric Colorimeter scale is specifically designed to enable the analyst to take full advantage of the validity of Beers Law for the colorimetric procedure being used. Because of the design of the Colorimeter and the use of highly selective light filters, it has been found that Beers Law is valid for practically all of the common colorimetric procedures under the conditions of this use in the Colorimeter. The scale is logarithmically spaced, not linearly spaced. Therefore, results are obtained by simple calculation from the scale reading, eliminating the need for calibration curves or semi-logarithmic paper.



**Spectral Transmission Curve for Filter No. 52**

For the majority of colorimetric procedures the reading will fall between 0 and 200 or 300. Readings above 500 should not be used as a basis for calculating results and solutions should be diluted accordingly.

**CALCULATION OF RESULTS USING A CALIBRATION FACTOR**

The logarithmic scale makes calculations very easy:

1. The reading of the unknown (corrected for a blank) is directly proportional to its concentration.
2. For best results, run a standard solution along with the unknown. Since the readings for both the standard and the unknown are proportional to the concentration, the results are calculated by use of the following formula:
3. Factor x reading of unknown = concentration of unknown.
4. The value of the factor is obtained from the scale reading for a solution of known concentration.
5. Factor =  $\frac{\text{Concentration of standard}}{\text{Reading of standard}}$

**TURBIDIMETRIC MEASUREMENTS**

The basis for calibration or calculation is based on a solution of standard turbidity, and the readings and results are obtained just as with clear solutions. To eliminate loss of determination due to the development of turbidity often the color is developed, the sample can be centrifuged in the macro test tubes using the ordinary 15ml brass centrifuge shield.

**ACCURACY**

Duplicate readings on the same solution have never been found to differ by more than 1/3 of 1 percent of the full scale. This corresponds to 1/3 of one scale division on the 100 to 0 scale of the usual Photoelectric Colorimeter.

# OPERATION

## IMPORTANT NOTES

- Make sure tubes are clean and dry. Wipe outside with a lint free tissue or cloth.
- The Colorimeter tubes must always be used with the lettering facing you. Do not use regular test tubes.
- Lamp may be left on all day, but should be shut off at night.
- DO NOT turn the lamp on without a filter in place. This could damage the photo cells.
- The cover over the cell compartment should be closed to keep out extraneous light.
- It is advisable to place the instrument on a table or bench free from vibration, and away from open doors, windows or strong overhead light.

## PROCEDURE

1. Make sure there is a filter in place between lamp housing and instrument (See Figure 1, page 3).
2. Pointer (C) on scale to be at 0. If not, adjust with small knob (D) on top of instrument.
3. Plug in instrument.
4. Place tube (E) with distilled water in place.
5. Turn large knob (A) on front of instrument to read 0 on scale (B).
6. Switch on Colorimeter lamp (F).
7. Readjust zero with larger knob (G) on top and further back on instrument.
8. Allow lamp to burn for a few minutes and check zero again.
9. The instrument is now ready for use.
10. To read unknown, remove distilled water and insert tube with unknown solution. The pointer (C) will be deflected from zero. Turn scale knob (A) until pointer has been brought back to zero. The reading on the scale (B) is now the reading of the unknown solution.
11. The concentration of the unknown is then obtained by multiplying scale reading by a factor of a known solution or by reading off a standard curve.

# MAINTENANCE

## CHANGING COLORIMETER LAMP

1. Disconnect instrument from line.
2. Turn instrument so that back faces you and remove back panel by sliding up.
3. Take note of which way the filament faces; the two loops near the bottom of the filament should face the lens.
4. Push lamp down and turn to left to remove.
5. Place new lamp in socket so the filaments will be properly oriented, push down and turn to the right.

## CARE OF SLIDE WIRE CONTACT

To keep dial contact clean, turn dial back and forth a number of times over the scale range by means of the large knob, taking care not to hit the end stops.

## KLETT CLINICAL COLORIMETER

### PARTS LIST

T37012-0000	Clinical Colorimeter, Tube Model 115V	800-3
T37012-0001	Clinical Colorimeter, Tube Model 220V	
T37012-0010	Test Tubes, calibrated, non-grad	801
T37012-0020	Test Tubes, calibrated, grad 5 - 1ml	802
T37012-1020	Klett Clinical Test Kit	
93701-2009	Lamp Bulb 110-120V/220-230V	802-L
37012-1000	Conversion kit 220 to 115V needed on older models for replacement bulbs	
93701-2103	Small Lamp for Dial 110-120V	6S6V
93701-2100	Small Lamp for Dial 220V	10S10
T37014-0040	KS-40 Filter	
T37014-0042	KS-42 Filter, Blue	803-B
T37014-0044	KS-44 Filter	
T37014-0047	KS-47 Filter	
T37014-0050	KS-50 Filter	
T37014-0052	KS-52 Filter	
T37014-0054	KS-54 Filter, Green	803-G
T37014-0055	KS-55 Filter	
T37014-0056	KS-56 Filter	
T37014-0059	KS-59 Filter	
T37014-0060	KS-60 Filter	
T37014-0062	KS-62 Filter	
T37014-0064	KS-64 Filter	
T37014-0066	KS-66 Filter, Red	803-R
T37014-0069	KS-69 Filter	
T37014-0070	KS-6225 Filter	
93701-2011	Extra frame for 2" Filter	808
93701-2012	Micro Test Tubes flat bottom, 2.5ml	802-M
93701-2014	Galvanometer	800-9
93701-2010	Photocells (pair)	801-15



# SUGAR (FOLIN-WU)

## THE DETERMINATION OF BLOOD SUGAR

Method of Folin and Wu, J. Biol. Chem. 41, 367 (1920)

**FILTER:** Use Filter 42 (Blue)

### PROCEDURE:

#### UNKNOWN

In Folin-Wu sugar tube:

2.0ml of 1:10 protein-free blood filtrate

2.0ml of alkaline copper reagent

Mix by lateral shaking and place in a boiling water bath for 6 minutes. Remove (without shaking) and cool in a large beaker of cold water for 2-3 minutes.

Add 2.0ml of phosphomolybdic acid color reagent.

Let stand for a few minutes until the cuprous oxide has completely dissolved, then dilute to the 25ml mark with distilled water. Mix well by repeated inversion, and allow to stand for 10-15 minutes. Transfer a portion of the colored solution to a colorimeter tube and read in the Colorimeter within the next 15 minutes, against a blank tube set at 0.

#### BLANK

Run a parallel determination as described above on 2.0ml of distilled water in place of the blood filtrate. Transfer a portion of the final solution to a colorimeter tube and set the Colorimeter to its 0 reading against this solution.

#### STANDARD

Run a determination as described above but use 2.0ml of a standard glucose solution instead of the blood filtrate. Read a portion of the final colored solution in the Colorimeter against the blank tube at 0.

### CALCULATION:

#### AGAINST THE STANDARD:

$$\frac{\text{Concentration of standard}}{\text{Reading of standard}} \times \text{reading of unknown} = \text{concentration of unknown}$$

A satisfactory standard for all blood sugar values up to about 400mg percent is the one corresponding to the 200mg percent standard used IN VISUAL COLORIMETRY. This standard contains 0.4mg of glucose in 2.0ml, and is prepared by diluting 2.0ml of the stock 1% glucose solution to 100ml with water. Take 2.0ml of this dilute standard for running the standard as described above. Since the dilute standard is the equivalent of a blood containing 200mg percent of blood sugar, use 200 as the concentration of standard in the formula above, and the result will be the blood sugar concentration of the unknown directly in mg percent.

#### USING A CALIBRATION FACTOR:

Reading of unknown x Folin-Wu blood sugar factor = mg percent blood sugar in unknown.

The factor is obtained from the reading of the standard solution as described above, and calculating the factor from the following formula:

$$\text{Folin-Wu blood sugar factor} = \frac{200}{\text{Reading of standard}}$$

The calibration factor should be determined in duplicate or triplicate and the average value taken. Once determined, the calibration factor is valid indefinitely within the limits of this type of calibration, as discussed above.

### REAGENTS:

#### ALKALINE COPPER REAGENT

Dissolve 40g of anhydrous sodium carbonate in about 400ml of distilled water, and transfer to a 1 liter volumetric flask.

Add 7.5g of tartaric acid, and when this has dissolved add 4.5g of crystallized copper sulfate. Grind to a fine powder and make up to the mark with distilled water. If a sediment forms in the bottle decant and use the clear supernatant fluid.

#### **PHOSPHOMOLYBDIC ACID COLOR REAGENT**

Place 35g of molybdc acid and 5g of sodium tungstate in a liter beaker. Add 200ml of 10% sodium hydroxide solution and 200ml of distilled water. Boil vigorously for 20 to 40 minutes. Cool, dilute to about 350ml and add 125ml of 85% (concentrated) phosphoric acid. Dilute to 500ml and mix.

#### **STANDARD GLUCOSE SOLUTIONS**

**Stock Standard:** Dissolve 1.0g of highest purity anhydrous dextrose in about 50ml of filtered saturated solution of benzoic acid in water, and make up to the 100ml mark of a volumetric flask with more of the saturated benzoic acid solution. This solution keeps indefinitely.

**Dilute Standard:** Transfer 2.0ml of the stock 1% glucose standard described above to a 100ml volumetric flask, and make up to the mark with water (or with saturated benzoic acid solution if the dilute standard will be kept for any length of time). Mix well. Of this solution, 2.0ml corresponds to a 1:10 filtrate of blood containing 200mg percent of blood sugar.

#### **SATURATED BENZOIC ACID SOLUTION**

In 1 liter volumetric flask, place 2.5g of benzoic acid and make up to 1000ml mark, with boiled distilled water.

### **PREPARATION OF A 1:10 PROTEIN-FREE FILTRATE**

Folin and Wu, J. Biol. Chem., 38, 81, (1919)

Place measured blood in a flask having 15 times the volume of the sample. Add 7 parts (7x the blood volume) of water, mix. Add 1 part 10% sodium tungstate solution, mix. Add slowly while shaking, 1 part 2/3N sulfuric acid. Shake in a stoppered flask and let stand for 10 minutes. Filter through a dry folded filter paper.

## **SUGAR (BENEDICT)**

### **THE DETERMINATION OF BLOOD SUGAR**

Method of Benedict, J. Biol. Chem., 76, 457 (1928)

**FILTER:** Use Filter 42 (Blue)

#### **PROCEDURE:**

##### **UNKNOWN**

In a Folin-Wu sugar tube:

2.0ml of a 1:10 protein-free blood filtrate

2.0 ml of copper reagent (containing bisulfite)

Mix by lateral shaking and place in a boiling water bath for 6 minutes. Remove (without shaking) and cool in a large beaker of cold water for two minutes. Add 2.0ml of the Benedict color reagent, mix by vigorous lateral shaking, and after about a minute add water to the 25ml mark. Mix well by repeated inversion, and allow to stand for about 10 minutes. Transfer a portion of the colored solution to a colorimeter tube and read in the Colorimeter against a blank tube set at 0.

##### **BLANK**

Run a parallel determination as described above on 2.0ml of distilled water in place of the blood filtrate. Transfer a portion of the final solution to a colorimeter tube and set the Colorimeter to its 0 reading against the solution.

##### **STANDARD**

Run a determination as described above but use 2.0ml of a standard glucose solution instead of the blood filtrate. Read a portion of the final colored solution in the Colorimeter against the blank tube at 0.

## **CALCULATION:**

### **AGAINST THE STANDARD**

$$\frac{\text{Concentration of standard}}{\text{Reading of standard}} \times \text{reading of unknown} = \text{concentration of unknown}$$

A standard corresponding to a 200mg percent blood sugar (see below) will give satisfactory results for all values up to about 500mg percent. If the value 200 is substituted for the concentration of the standard in the above formula, the result will give directly the blood sugar content of the unknown in mg percent.

### **USING A CALIBRATION FACTOR:**

Reading of unknown x Benedict blood sugar factor = mg percent blood sugar in unknown.

The factor is obtained from the reading of the standard solution as described above:

$$\text{Benedict blood sugar factor} = \frac{200}{\text{Reading of standard}}$$

The calibration factor should be determined in duplicate or triplicate and the average value taken. Once determined, the calibration factor is valid indefinitely within the limits of this type of calibration, as discussed above.

## **REAGENTS:**

### **COPPER REAGENT**

Dissolve 15g of anhydrous sodium carbonate, 3g of alanine, and 2g of Rochelle salt in about 250ml of distilled water. In another beaker dissolve 3g of crystalline copper sulfate in about 100ml of distilled water. Add this solution to the first solution while stirring, and dilute to 500cc. This reagent is stable for 4-6 weeks. If mold grows in the solution during this time pour the solution through a small piece of cotton in a funnel.

### **COPPER REAGENT CONTAINING BISULFITE**

To 20ml of the copper reagent described above add 1ml of 1% sodium bisulfite solution and mix. The bisulfite solution should be prepared fresh once a month.

The reagent with bisulfite is stable for one or two days only, and only enough for immediate needs should be prepared. Smaller or larger quantities than that cited above may be prepared using proportionate amounts of the two solutions.

### **BENEDICT COLOR REAGENT**

To 150g of pure molybdc acid and 75g anhydrous sodium carbonate in a large flask, add cautiously and in small portions about 500ml of distilled water, while shaking. Heat to boiling and filter. Wash residue on filter until filtrate and washings have a volume of about 600ml. Very slowly add 300ml of 85% (concentrated) phosphoric acid, cool, and dilute to 1 liter.

### **STANDARD GLUCOSE SOLUTIONS**

The glucose standards described under the Folin-Wu blood sugar method may be used for this procedure.

The working standard should be the one corresponding to a 200mg percent blood sugar.

## **SUGAR (FOLIN AND MALMROS)**

### **MICRO-METHOD FOR THE DETERMINATION OF BLOOD SUGAR**

Method of Folin and Malmros, J. Biol. Chem., 83, 115 (1929)

**FILTER:** Use Filter 54 (Green)

### **PROCEDURE:**

#### **UNKNOWN**

Collect 0.1ml of blood from the finger tip or ear in a pipette calibrated to contain 0.1ml. Transfer to 10.0ml of

dilute tungstic acid solution in a 15ml centrifuge tube, rinsing out the pipette several times with portions of the solution in the centrifuge tube. Stir well with the top of the pipette while rinsing it out, remove the pipette, and centrifuge the contents of the tube for a few minutes.

Place 4.0ml of the water-clear supernatant fluid in a test tube graduated at 25ml. Add 2ml of the potassium ferricyanide solution, followed by 1ml of the cyanide-carbonate solution, mix by lateral shaking, and place in a boiling water bath for 8 minutes. Cool by placing in a large beaker of cold water for 1-2 minutes, then add 5ml of the ferric iron-gum ghatti solution and mix by shaking. Let stand for a few minutes and then dilute to the mark with distilled water. A few drops of alcohol may be added before diluting quite to the mark to cut the foam if desired. After diluting to the mark, mix well by inversion, and allow to stand for 10 minutes.

Transfer a portion of the colored solution to a colorimeter tube and read in the Colorimeter against the blank tube set at 0 within the next 30 minutes.

#### **BLANK**

Place 4.0ml of distilled water in a test tube graduated at 25ml and add the ferricyanide and cyanide-carbonate solutions exactly as described above for the unknown. Heat in the water bath along with the unknown, cool and develop the color as described above. Transfer a portion of the final colored solution to a colorimeter tube, place in the Colorimeter and set the instrument to its 0 reading against the blank.

#### **STANDARD**

Place 4.0ml of standard glucose solution in a test tube graduated at 25ml, add the ferricyanide and cyanide-carbonate solutions and continue the colorimetric procedure exactly as described above. Transfer a portion of the final colored solution to a colorimeter tube and read against the blank set at 0, following the time conditions specified for the unknown.

#### **CALCULATION**

##### **AGAINST THE STANDARD:**

$$\frac{200}{\text{Reading of standard}} \times \text{reading of unknown} = \text{mg percent blood sugar in original blood}$$

The standard is the equivalent of a blood containing 200mg percent of blood sugar. Other standards may be used if the proper value is substituted in the formula above. The proportionality is excellent for all values of blood sugar up to 500mg percent.

##### **USING A CALIBRATION FACTOR:**

Reading of unknown x Folin-Malmros blood sugar factor = mg percent blood sugar in original blood.

The value of the factor is obtained from the reading of a standard solution as described above:

$$\text{Folin-Malmros blood sugar factor} = 200/\text{reading of standard} \frac{200}{\text{Reading of standard}}$$

The value of the factor should be determined in duplicate or triplicate and the average taken. Once determined, the calibration factor is valid indefinitely within the limits of this type of calibration as discussed above.

#### **REAGENTS:**

##### **DILUTE TUNGSTIC ACID SOLUTION**

Dilute 20ml of 10% sodium tungstate solution to about 800ml in a 1 liter volumetric flask. Add while shaking 20ml of 2/3N sulfuric acid, and dilute to the mark. Mix well, and store away from light. If this solution no longer gives water-clear supernatants fluids when used as described for the precipitation of blood proteins it should be discarded.

##### **POTASSIUM FERRICYANIDE SOLUTION**

Dissolve 2g of high grade potassium ferricyanide (free from ferricyanide) in distilled water and dilute to 500ml. Keep in a brown bottle away from light. Remove small portions for daily use, keeping these also in a brown bottle.

##### **CYANIDE-CARBONATE SOLUTION (POISONOUS)**

Dissolve 8g of anhydrous sodium carbonate in about 50ml of distilled water in a 500ml volumetric flask by shaking. Add 150ml of freshly prepared 1% sodium cyanide solution, mix and dilute to volume. Mix well and store in a clean

bottle. Always dispense this solution from a burette. This solution should last for several months.

#### **FERRIC IRON-GUM GHATTI SOLUTION**

Suspend 20g of soluble gum ghatti by means of a wire screen or cloth bag just below the surface of a liter of cold distilled water in a cylinder. Leave 18 hours or longer, remove the bag or screen and strain the fluid in the cylinder through a double layer of clean towel. Dissolve 5g of anhydrous ferric sulfate in a mixture of 75ml of 85% phosphoric acid and 100ml of distilled water, and add this solution to the gum ghatti solution. Mix well. Add to the mixture about 15ml of a 1% potassium permanganate solution, avoiding an excess as evidenced by a permanent pink color. This solution appears to keep indefinitely. If turbid, the solution may be placed in an incubator at 37 degrees C for a few days.

#### **STANDARD GLUCOSE SOLUTION**

A standard glucose solution corresponding to a 200mg percent blood sugar as determined by this method contains 0.02mg of glucose per ml. It may be prepared by diluting exactly 2.0ml of a stock 1% solution of glucose in saturated benzoic acid solution (see the Folin-Wu blood sugar method) to 1 liter with distilled water. This dilute standard should be prepared fresh daily from the stock solution, which keeps indefinitely.

## **NON-PROTEIN NITROGEN**

### **THE DETERMINATION OF NON-PROTEIN NITROGEN IN BLOOD**

Method of Koch and McMeekin, J. Am. Chem. Sol., 46, 2066 (1924)

**FILTER:** Use Filter 54 (Green)

#### **PROCEDURE:**

##### **UNKNOWN:**

In a large pyrex test tube graduated at 50ml:

5.0ml of the 1:10 protein-free blood filtrate

1 ml of 1:1 sulfuric acid

1 or 2 clean glass beads

Heat over a microburner until the water has been driven off and dense white fumes fill the tube. With the tube in a upright position drop directly into the solution 1 to 3 drops of 30% hydrogen peroxide solution. Heat again to boiling. The solution should become colorless - if not, repeat the addition of the hydrogen peroxide and boiling. After decolorization boil the solution gently for about 5 minutes. Cool and dilute to the 50ml mark with distilled water. Mix well by inversion.

Transfer a 10.0ml portion of the diluted solution to a colorimeter tube in which 2 drops of gum ghatti solution have already been placed. Add 3.0ml of the Koch McMeekin Nessler reagent, mix by inversion and allow to stand for 10 minutes. Read in the Colorimeter within the next 20 minutes, against the blank tube or distilled water (see below) set at 0.

##### **BLANK:**

In a pyrex test tube (or volumetric flask) graduated at 50ml place 1ml of the 1:1 sulfuric acid solution and dilute to the mark. Mix well, and transfer a 10.0ml portion to a colorimeter tube containing 2 drops of gum ghatti solution. Add 3.0ml of the Nessler solution, mix by inversion, and allow to stand for 10 minutes. Read in the Colorimeter within the next 20 minutes, against a distilled water 0.

The value of the blank in this procedure includes the ammonia present in the reagents, except for the hydrogen peroxide. This should be nitrogen-free, as indicated below. Since the blank is ordinarily quite small and constant, it is satisfactory to determine it once for a given lot of reagents, and to read future unknowns against a distilled water 0 rather than a blank 0, subtracting the value of the blank 0, subtracting the value of the blank (if significant) from each unknown reading in order to obtain the true reading of the unknown.

##### **STANDARD:**

In a pyrex test tube (or volumetric flask) graduated at 50ml, place sufficient standard ammonium sulfate solution

to contain 0.25mg of nitrogen. Add 1 ml of the 1:1 sulfuric acid and dilute with distilled water to the mark. Mix well, and transfer 10.0ml portions of this solution to colorimeter tubes containing 2 drops of gum ghatti solution, followed by 3.0ml of Nessler solution as described above. Read between 10 and 30 minutes after the addition of the Nessler reagent against distilled water set at 0.

Subtract the value of the blank to obtain the true reading of the standard.

#### **CALCULATION:**

##### **AGAINST THE STANDARD:**

$$\frac{50}{\text{Reading of standard}} \times \text{reading of unknown} = \text{mg percent N.P.N. in original blood}$$

The standard described above corresponds to a 1:10 filtrate of blood containing 50mg percent N.P.N. Another standard may be used if the proper value is substituted in the formula above. The proportionality is satisfactory up to about 80mg percent N.P.N. if the reading is higher than this, the determination should be repeated on a diluted portion of the filtrate.

##### **USING A CALIBRATION FACTOR:**

Reading of unknown x blood N.P.N. factor = mg percent N.P.N. in original blood.

The factor is obtained from the reading of a standard solution as described above:

$$\text{blood N.P.N. factor} = \frac{50}{\text{Reading of standard}}$$

The calibration factor should be determined in duplicate or triplicate and the average value taken. Once determined, the calibration factor is valid indefinitely within the limits of this type of calibration, as discussed above.

#### **REAGENTS:**

##### **1:1 SULFURIC ACID**

Dilute one volume of concentrated sulfuric acid with an equal volume of distilled water. Cool and store in a well stoppered bottle.

##### **30% HYDROGEN PEROXIDE SOLUTION (WAX SEAL)**

This must be nitrogen-free. Satisfactory preparations are commercially available. To test for the presence of nitrogen, run a complete determination as described for the blood filtrate above, but use 5.0ml of distilled water containing a known (e.g. 10 drops) of the hydrogen peroxide, omitting the further addition of hydrogen peroxide after the stage of white fumes is reached, unless the solution is discolored at this point. If more peroxide is needed at this point the added amount should be included in the calculations. After dilution of the digest to the mark, develop the color on a 10ml portion of the solution exactly as described above, reading against distilled water at 0. Subtract the value of the blank from the reading to get the correction in scale reading for the known number of drops of hydrogen peroxide, from which the correction per drop may be made. The chief purpose of this determination is to guard against the use of a contaminated lot of hydrogen peroxide. If the correction is significant the hydrogen peroxide should be discarded.

##### **GUM GHATTI SOLUTION**

Fill a liter graduated cylinder to the mark with cold distilled water, and suspend just below the surface 20g of soluble gum ghatti by means of a wire screen or cloth bag. Allow to stand 18 hours or longer, remove the undissolved material and filter the solution through coarse filter paper or strain through a clean towel.

##### **KOCH-MCMEEKIN NESSLER SOLUTION**

Dissolve 30g of potassium iodide in 20ml of distilled water and allow to stand 18 hours or until completely dissolved. Add 22.5g of iodine to the solution. Shake until dissolved, then add 30g of pure metallic mercury. Shake the mixture well, keeping the solution cool by holding under running tap water from time to time, until the supernatant liquid had lost its yellow color. Pour off from the undissolved mercury and test for the presence of excess iodine by adding a few drops to starch solution in a test tube. If no blue color is obtained, add iodine solution similar to that described above, drop by drop, until there is a faint excess of free iodine as determined by testing a few drops with starch solution. Dilute to 200ml, mix and pour

into 975ml of accurately prepared 10% sodium hydroxide solution. Mix well and allow any precipitate to settle out, using the clean supernatant fluid. Avoid stirring up the sediment when removing from the storage bottle.

### **STANDARD NITROGEN SOLUTION**

A standard solution containing 1 mg of nitrogen in 10ml is the ordinary standard for nitrogen determinations, and is prepared by dissolving 0.4716g of highest purity ammonium sulfate in ammonia-free distilled water, adding about 1 ml of concentrated sulfuric acid, and diluting to the mark in a liter volumetric flask with ammonia-free distilled water. Exactly 2.5ml of this solution contains 0.25mg of nitrogen, the amount specified in the directions above.

## **URIC ACID**

### **THE DETERMINATION OF URIC ACID IN BLOOD**

Method of Folin, J. Biol. Chem., 101, 111 (1933); 106; 311 (1934)

**FILTER:** Use Filter 54 (Green)

#### **PROCEDURE:**

##### **UNKNOWN:**

In a colorimeter tube:

1.0ml of a 1:10 protein-free blood filtrate

2.0ml of urea-cyanide solution (from a burette)

0.8ml of uric acid reagent

Mix by lateral shaking and allow to stand for 20 minutes. Dilute to the 10ml mark with distilled water, mix well by inversion, and read in the Colorimeter within the next 20 minutes or so against the blank set at 0.

##### **BLANK:**

Run a determination as described above, but use 1.0ml of distilled water instead of the blood filtrate. Place in the Colorimeter and adjust to 0 reading.

##### **STANDARD**

Run a determination as described above, but use 1.0ml of standard uric acid solution containing 0.004mg of uric acid per ml. Read in the Colorimeter against the blank at 0.

#### **CALCULATION:**

##### **AGAINST THE STANDARD:**

$$\frac{4.0}{\text{Reading of standard}} \times \text{reading of unknown} = \text{mg percent uric acid in original sample}$$

The standard described above corresponds to a 1:10 filtrate of blood containing 4.0mg percent uric acid. Another standard may be used if the proper value is substituted in the formula above. The proportionality is good up to 10mg percent.

##### **USING A CALIBRATION FACTOR:**

Reading of unknown x Folin blood uric acid factor = mg percent uric acid in unknown.

The factor is obtained from the reading of a standard solution as described above.

$$\text{Folin blood uric acid factor} = \frac{4.0}{\text{Reading of standard}}$$

The calibration factor should be determined in duplicate or triplicate and the average value taken. Once determined, the calibration factor is valid indefinitely within the limits of this type of calibration, as discussed above. Watch the reagent blank in this determination, and if it is high discard the reagent.

## REAGENTS:

### UREA-CYANIDE SOLUTION (POISONOUS)

Place 75g of good quality sodium cyanide in a 2 liter beaker, and add 700ml of distilled water. Stir until the cyanide is completely dissolved. Add 300g of urea and stir, followed by 4 to 5g of calcium oxide, stirring for about 10 minutes. Let stand 24 hours and filter. Add about 2g of powdered lithium oxalate to the filtrate, shake occasionally for 10 to 15 minutes, and filter. This solution should be handled with care, and should always be dispensed from a burette.

### URIC ACID REAGENT

Place 100g of molybdate-free sodium tungstate (Folin's Sodium Tungstate) in a 500ml Florence flask. To 150ml of water in another vessel add 32 to 33ml of 85% (concentrated) phosphoric acid. Mix and pour into the flask containing the tungstate. Add a few quartz pebbles and heat to boiling with a Meeker burner, then boil gently over a microburner for 1 hour, with a funnel in the neck of the flask carrying a 200ml flask filled with cold water to act as a condenser for escaping water vapor.

After the 1 hour boiling period add a few drops of bromine water to decolorize, boil off the excess bromine, cool, and dilute to 500ml. If this reagent gives too high a blank add 3 to 5 (but not more) of sodium tungstate and boil for another 10 to 15 minutes. Decolorize with bromine water as before.

### STANDARD URIC ACID SOLUTIONS

**Stock Standard:** In a 250ml flask, dissolve by shaking 0.6g of lithium carbonate in 150ml of distilled water, and filter. Warm the filtrate (not necessarily entirely clear) to 60 degrees C. Weigh out exactly 1g of pure uric acid on a watch glass and transfer as much as possible by means of a medium size funnel to a 1 liter volumetric flask, tapping the funnel with the watch glass. Leave the glass in the funnel in the neck of the flask, and warm the flask under running warm water. Pour the warm lithium carbonate solution into the flask, rinsing down the traces of uric acid remaining on the funnel and watch glass. Shake the warm solution so as to dissolve the uric acid promptly. It should not take more than about 5 minutes to dissolve the uric acid (remember the lithium carbonate solution itself may be slightly turbid). After solution of the uric acid, cool the flask by shaking under running cold water. Add 20ml of 40% formalin, and half fill the flask with distilled water. Add a few drops of methyl orange solution. Then, while shaking, add slowly from a pipette 25ml of normal sulfuric acid. The total acidity should be such that the methyl orange turns pink when there are about 2 or 3ml of acid still left in the pipette. Dilute to the 1 liter mark, mix well, and store in a clean, tightly stoppered brown glass bottle and keep away from light. This solution appears to last for years, and contains 1mg of uric acid per ml.

**Working Standards:** Dilute 1.0ml of the stock standard with distilled water to 250ml. This solution keeps for days and contains 0.004mg of uric acid per ml.

## UREA

### THE DETERMINATION OF UREA IN BLOOD

Method of Karr: J. Lab. Clin. Med., 9, 329 (1924)

**FILTER:** Use Filter 54 (Green)

### PROCEDURE:

#### UNKNOWN:

In a test tube graduated at 25ml.:

5.0ml of a 1:10 protein-free blood filtrate

1 drop of buffer solution

5 drops of urease solution or 1 drop of Koch's glycerol-urease extract

Mix gently and place in a water bath or incubator at 50 degrees C for 15 minutes. At the end of this time, dilute to the 25ml mark with distilled water and mix well.

Transfer 10.0ml of the solution to a colorimeter tube in which 2 drops of gum ghatti solution have already been placed. Add 1.0ml of Koch-McMeekin Nessler solution, mix by inversion, and allow to stand for 10 minutes.

Read in the Colorimeter within the next 20 minutes, against a 0 of distilled water. Subtract the reading of the blank from the reading of the unknown to obtain the true reading of the unknown.



**BLANK:**

This will correct for the ammonia in the reagents, particularly in the urease solution, which should be made fresh daily if the jack-bean extract is used (see below). Koch's glycerol-urease extract is said to last a year. Run a complete procedure as described above, using 5.0ml of distilled water instead of blood filtrate. Read the final colored solution in the Colorimeter against distilled water at 0.

The blank reading will probably be small, and reasonably consistent. If so, the value of the blank as determined at one time may be subtracted from future readings of unknowns without a separate determination.

**STANDARD**

Run a determination as described above on 5.0ml of a standard urea solution containing the equivalent of 45mg percent of urea nitrogen (see above). Read the final colored solution in the Colorimeter against distilled water at 0. Subtract the value of the blank from the reading of the standard to obtain the true reading of the standard.

**CALCULATION:****AGAINST THE STANDARD:**

$$\frac{45}{\text{Reading of standard}} \times \text{reading of unknown} = \text{mg percent blood urea nitrogen in original blood}$$

The standard described below contains the equivalent of 45 mg percent of blood urea nitrogen with a 1:10 filtrate. Other standards may be used if proper value is substituted in the formula above. The proportionality is good up to about 80 to 90mg percent. If the filtrate reads higher than this, the determination should be repeated on a diluted portion.

**USING A CALIBRATION FACTOR:**

Reading of unknown x blood urea nitrogen factor = mg percent blood urea nitrogen in original blood.

The factor is obtained from the reading of a standard solution as described above.

$$\text{Blood urea nitrogen factor} = \frac{45}{\text{Reading of standard}}$$

The value of the factor should be determined in duplicate or triplicate and the average value taken. Once determined, the calibration factor is valid indefinitely within the limits of this type of calibration, as discussed above.

**REAGENTS:****BUFFER SOLUTION: (FOR UREA)**

Dissolve 14g of crystalline sodium pyrophosphate (containing 10 molecules of water) in sufficient N/2 phosphoric acid to make 100ml. The N/2 phosphoric acid is prepared by diluting 17ml of 85% phosphoric acid to 1 liter and titrating 5ml portions against N/10 alkali to a faint pink color with phenolphthalein. On the basis of this titration the phosphoric acid can be diluted to give a substantially correct N/2 solution.

**UREASE SOLUTION:**

Treat 0.5g of jack bean meal in a small flask with 20ml of 30% by volume alcohol. Shake for ten minutes and filter or centrifuge. The extract should be prepared on the day of use.

**GLYCEROL-UREASE SOLUTION:**

The directions for preparing this solution may be found in Koch: J. Lab. Clin. Med., 11, 776 (1926).

**GUM GHATTI SOLUTION AND KOCH-MCMEEKIN NESSLER SOLUTION**

See: The determination of non-protein nitrogen in blood, above.

**STANDARD UREA SOLUTIONS:**

**Stock Standard:** Dissolve 0.643g of pure urea in distilled water, and make up to the mark in a 1 liter volumetric flask. This solution contains 0.3mg of urea nitrogen per unit.

**Working Standard:** Dilute 15.0ml of the stock standard described above to 100ml with distilled water, and mix. 5.0ml of this solution corresponds to a 1:10 blood filtrate for a blood containing 45mg percent of urea nitrogen.

# CREATININE

## THE DETERMINATION OF CREATININE IN BLOOD

**FILTER:** Use Filter 54 (Green)

### PROCEDURE:

#### UNKNOWN:

5.0ml of a 1:10 protein-free blood filtrate.

2.5ml alkaline picrate solution.

Mix by inversion.

Read in the Colorimeter between 10 and 20 minutes after the addition of the picrate, against the blank tube set at 0.

#### BLANK:

5.0ml distilled water

2.5ml alkaline picrate solution

Mix by inversion.

Place in the Colorimeter and adjust to 0 reading.

#### STANDARD

5.0ml of standard creatinine solution. Containing 0.03mg of creatinine in 5ml.

2.5ml alkaline picrate solution

Mix by inversion.

Read in the Colorimeter between 10 and 20 minutes after the addition of the picrate, against the blank tube set at 0.

### CALCULATION:

#### AGAINST THE STANDARD:

$$\frac{6.0}{\text{Reading of standard}} \times \text{reading of unknown} = \text{mg percent of creatinine in original blood}$$

The standard corresponds to 6.0mg percent of blood creatinine in a 1:10 filtrate of whole blood. The proportionality is good over the range from 0 to 10mg percent. The standard is the same one that is ordinarily used in the colorimetric determination of blood creatinine. Other standards may be used if the proper value is substituted in the calculations.

#### USING A CALIBRATION FACTOR:

Reading of unknown x blood creatinine factor = mg percent blood creatinine is unknown.

The factor is obtained by reading the standard solution described above, and calculating the factor from the following formula:

$$\text{blood creatinine factor} = \frac{6.0}{\text{Reading of standard}}$$

The calibration factor should be determined in duplicate or triplicate and the average value taken. Once determined, the calibration factor is valid indefinitely within the limits of this type of calibration, as discussed above.

### REAGENTS:

#### ALKALINE PICRATE SOLUTION:

To five volumes of a saturated solution (1.2%) of purified picric acid add one volume of 10% sodium hydroxide solution and mix. This solution should be freshly prepared.

#### STANDARD CREATININE SOLUTIONS:

**Stock Standard:** Dissolve 0.100g of pure creatinine in 100ml of N/10 hydrochloric acid. This solution contains 1mg of creatinine in 1 ml.

**Dilute Standard:** Transfer 6.0ml of the stock standard creatinine solution to a liter volumetric flask, add 1 ml of concentrated hydrochloric acid, dilute to the mark and mix well. This solution contains 0.03mg of creatinine in 5ml. When used as described above it is the equivalent of a 1:10 blood filtrate from a blood sample containing 6.0mg percent of creatinine.

## BILIRUBIN

### THE DETERMINATION OF SERUM BILIRUBIN

Method of Malloy and Evelyn, J. Biol. Chem., 119, 481 (1937)

**FILTER:** Use Filter 54 (Green)

**PROCEDURE:**

Dilute 1.0ml of serum or plasma to 10.0ml with distilled water and mix. Prepare three colorimeter tubes as follows:

**UNKNOWN:**

5.0ml of absolute methyl alcohol and 1.0ml of diazo reagent.

**BLANK:**

5.0ml of absolute methyl alcohol and 1.0ml of diazo blank solution.

**STANDARD:**

5.0ml of bilirubin standard in absolute methyl alcohol and 1.0ml of diazo reagent.

Add a 4.0ml portion of the diluted serum to the blank tube and the unknown tube, and a 4.0ml portion of distilled water to the standard tube. Mix by gentle inversion, taking care to treat the blank and unknown tubes as uniformly as possible. Allow to stand at room temperature for 30 minutes to permit maximum color development.

When the tubes are ready to read in the Colorimeter, remove any bubbles which may be present by tapping the tubes on a wooden block. Read in the Colorimeter against a distilled water 0. Subtract the reading of the blank tube from the reading of the unknown tube, to obtain the true reading of the unknown.

**CALCULATION:**

The concentration of bilirubin in the sample is obtained as follows:

$$\frac{\text{mg bilirubin in 5.0 ml standard}}{\text{Reading of standard}} = \text{mg bilirubin in the portion of sample analyzed}$$

Since the portion of sample analyzed represents 0.4ml of serum, the results as obtained above must be multiplied by 250 to obtain the serum bilirubin content of the original sample in mg percent. If the standard described below is used (equivalent to 5ml percent serum bilirubin), the calculation becomes:

$$5.0/\text{reading of standard} \times \text{reading of unknown} = \frac{5.0}{\text{Reading of standard}}$$

**USING A CALIBRATION FACTOR:**

Malloy and Evelyn use a calibration factor rather than compare the unknown against a simultaneously prepared standard as described above, since they find the reading for a known amount of pure bilirubin to be quite reproducible. A calibration factor may be obtained as follows: Treat duplicate or triplicate 5.0ml portions of the 5mg percent standard described below with 1.0ml of the diazo reagent and 4.0ml of distilled water. Mix by inversion and allow to stand for 30 minutes or until maximum color development has been obtained. Read in the Colorimeter against a distilled water 0. From the average reading of the standard and its equivalent concentration the calibration factor is obtained as follows:

$$\frac{5.0}{\text{Reading of standard}} = \text{calibration factor}$$

Once determined accurately, the calibration factor is valid indefinitely

within the limits of this type of calibration, as discussed above. Calculations using the calibration factor are carried out as follows:

Reading of unknown x calibration factor = mg percent serum bilirubin

**REAGENTS:**

**METHYL ALCOHOL, absolute, c.p. (metal capped)**

**DIAZO BLANK SOLUTION:**

Dilute 15ml of concentrated hydrochloric acid to 1 liter with distilled water.

**DIAZO REAGENT:**

Made up fresh as needed from two stock solutions, prepared as follows:

**Solution A:** Dissolve 1g of sulfanilic acid in 15ml of concentrated hydrochloric acid, and dilute to 1 liter with distilled water.

**Solution B:** Dissolve 0.5g of c.p. sodium nitrite in a little water and dilute to 100ml in a graduate.

Solution A will keep indefinitely. Solution B should be made fresh daily.

To prepare the diazo reagent, add 3ml of solution B to 100ml of solution A and mix. The mixed solution should be used shortly after preparation. Smaller or larger quantities of the diazo reagent may be prepared using the proportional amounts of solutions A and B given above.

**BILIRUBIN STANDARD**

**Stock Standard:** Dissolve 40mg of pure bilirubin in pure chloroform and dilute to 100ml with chloroform. This stock standard, stored in a brown glass bottle, should keep indefinitely. It contains 0.4mg of bilirubin per ml. Known amounts of the stock standard are diluted with methyl alcohol to give working standards.

**Working Standard:** A satisfactory working standard contains 0.02mg bilirubin in 5.0ml, and is prepared by diluting 1.0ml of the stock standard to 100ml with methyl alcohol. This standard corresponds to a 5mg percent serum bilirubin when used as described above.

## CHOLESTEROL

### THE DETERMINATION OF CHOLESTEROL IN WHOLE BLOOD, PLASMA OR SERUM

Modification of the method of Bloor, Pelkan and Allen, J. Biol. Chem., 52, (1992)

**FILTER:** Use Filter 42 (Blue)

**PROCEDURE:**

**UNKNOWN AND BLANK:**

Place about 20ml of alcohol-ether mixture in a 25ml glass-stoppered flask. Slowly add, while rotating, exactly 0.5ml of the whole blood, plasma or serum to be analyzed. The resulting precipitate should be finely divided and not lumpy. Immerse the flask in boiling water, shaking gently to prevent bumping, until the contents of the flask have boiled for a few seconds. Remove the flask and cool to room temperature. Make up to 25ml mark with alcohol-ether mixture, stopper and shake well. Pour onto a dry fat-free filter paper, collecting the filtrate in a dry flask.

Transfer 5.0ml of the filtrate to a small dry beaker and evaporate to dryness on a hot plate or water-bath, add a 1ml portion of anhydrous chloroform to the dry residue and bring to a momentary boil. Carefully pour off the liquid into a clean dry colorimeter tube. Repeat this process with two more 1ml portions of chloroform, in each case transferring the extract carefully to the colorimeter tube. Allow the combined extracts in the colorimeter tube to cool to room temperature and make up to a final volume of 5.0ml with anhydrous chloroform.

Before proceeding with the analysis, prepare a blank tube as follows: place 5.0ml of anhydrous chloroform in a clean dry colorimeter tube and add 1.0ml of acetic anhydride (from a burette). Mix with a dry glass rod, remove the rod, place the tube in the Colorimeter and adjust the instrument to its 0 reading.

Now add to the contents of the unknown exactly 1.0ml of acetic anhydride, mix with a dry glass rod, remove the rod and read in the Colorimeter against the blank tube at 0. The reading of the unknown at this time is called the R, reading, and is to correct for any color present before the cholesterol color is developed. After

obtaining this reading, put the stirring rod back in the colorimeter tube and set it aside until the standard has been prepared.

**STANDARD:**

Place 5.0ml of a standard solution of cholesterol in chloroform in a clean dry colorimeter tube and add 1.0ml of acetic anhydride. Insert a glass rod and mix.

Now to both standard and unknown add 0.1ml of concentrated sulfuric acid. Mix well with the glass rods and then set aside in a dark place for 15 minutes. At the end of this time remove the rods and read the tubes in the Colorimeter within the next 15 to 20 minutes against the blank tube set at 0. The reading of the unknown tube at this time is called the  $R_2$  reading. The cholesterol content of the unknown is obtained from the standard and unknown readings as follows:

$$\frac{\text{mg cholesterol in 5.0 ml standard}}{\text{Reading of standard}} \times (R_2 - R_1) = \text{mg cholesterol in 5.0ml of alcohol-ether filtrate}$$

Since the 5.0 ml portion of alcohol-ether filtrate taken for analysis represents 0.1ml of the original sample, the value obtained by the equation above is multiplied by 1000 to give the cholesterol content of the original sample in milligrams percent.

**REAGENTS:**

**ALCOHOL-ETHER MIXTURE: (METAL CAPPED)**

To 3 parts of 95% ethyl alcohol add 1 part of high grade ether and mix.

**CHLOROFORM, ACETIC ANHYDRIDE, SULFURIC ACID:**

These reagents must be of the highest quality. It is particularly important that the chloroform be specifically anhydrous. Ordinary chloroform, or old and deteriorated material will lead to weak and uncertain colors.

**STANDARD CHOLESTEROL SOLUTIONS:**

**Stock Standard:** This is made up from c.p. cholesterol and anhydrous chloroform and kept in the refrigerator. A satisfactory stock standard contains 160mg cholesterol in 100ml chloroform.

**Working Standard:** Dilute 1.0ml of stock standard to 25ml with anhydrous chloroform in a glass stoppered volumetric flask. This standard contains 0.32mg of cholesterol in 5.0ml. It keeps well for some days in the refrigerator, and is equivalent to 320mg percent of blood cholesterol when the blood filtrate is prepared as described above.

## VITAMIN C

### THE DETERMINATION OF ASCORBIC ACID (VITAMIN C) IN PLASMA

Method of Mindlin and Butler, J. Biol. Chem, 122, 673, (1938)

**FILTER:** Use Filter 54 (Green)

**PROCEDURE:**

**UNKNOWN:**

Place one drop each of 20% potassium oxalate and 5% potassium cyanide solution in a test tube, and add 4-5ml of the freshly drawn blood sample. Mix, transfer to a centrifuge tube and centrifuge. Add 2.0ml of the plasma so obtained to 2.0ml of distilled water in a test tube, followed by 4.0ml of 5% metaphosphoric acid. Shake gently and pour onto a dry filter.

Place 4.0ml of indophenol-acetate solution in a colorimeter tube, add 4.0ml of the plasma filtrate obtained as described above, mix and read immediately in the Colorimeter against a distilled water 0.

The reading should be made 30 seconds after the addition of the filtrate to the dye. Mindlin and Butler suggest additional readings 1, 2, and 3 minutes after the addition of the filtrate, to enable correction (by extrapolation) to be made for the possible presence of other and more slowly reacting reducing substances than ascorbic acid, but state that this correction is usually insignificant.

**BLANK:**

Measure a 4.0ml portion of the same indophenol-acetate solution that was used for the unknown into a colorimeter tube, add 4.0ml of 2.5% metaphosphoric acid solution, mix and read immediately in the Colorimeter, against a distilled water 0.

**CALCULATION:**

The difference in reading between the blank and the unknown is the measure of the ascorbic acid concentration of the sample. To calculate results, it is necessary to know what this difference is for a known amount of ascorbic acid. This determines the value of the plasma ascorbic acid factor. When the value of the factor is known, the concentration of unknown is obtained as follows:

(Blank reading - unknown reading) x plasma ascorbic acid factor = mg percent ascorbic acid in plasma

The value of the factor is obtained by running a colorimetric determination as described above (including the blank) on 4.0ml of a standard solution of ascorbic acid, instead of the plasma filtrate. If the standard is equivalent to 2.0mg percent plasma ascorbic acid, as described below, the value of the factor is obtained as follows:

$$\frac{2.0}{\text{Blank reading} - \text{Standard reading}} = \text{plasma ascorbic acid factor}$$
Once the value of the factor is determined, it

should be valid indefinitely for all the indophenol-acetate solutions that are prepared from the particular lot of dye at hand. When starting a new lot of dye it may be necessary to re-standardize the procedure.

Since the procedure depends only on a difference between the blank and the unknown, and a given amount of ascorbic acid will always give the same difference in readings, regardless of the actual numerical value of the readings themselves, it is relatively unimportant what the reading of the dye blank is. It should not be too low, otherwise there may be more than enough ascorbic acid in the unknown to reduce the dye completely, nor should it be too high, since this will reduce the accuracy of the procedure. A reading of the dye blank of around 150 or so should be satisfactory for most purposes. The dye blank need not be the same for the unknown as for the standard when the calibration factor was obtained, or even for two separate unknowns, but it is essential that an unknown reading and its blank be based on the same dilute dye solutions.

**REAGENTS:****5% POTASSIUM CYANIDE, 20% POTASSIUM OXALATE:**

Prepare on the indicated basis from high grade material and distilled water.

**5% METAPHOSPHORIC ACID:**

Dissolve 10g of reagent quality metaphosphoric acid in distilled water and dilute to 200ml. Keep in the refrigerator. This solution should keep for two weeks.

**INDOPHENOL-ACETATE SOLUTION:**

Dissolve a few crystals of 2, 6-dichlorophenol indophenol (Sodium 2, 6-dichloro-benzenoneindophenol) in a small amount of warm distilled water, filter, cool the filtrate and dilute with distilled water to such a strength that when a small portion of the dilute dye is mixed with an equal volume of the buffered sodium acetate solution described below, the resulting solution reads around 150 or so in the photoelectric Colorimeter against a distilled water 0. Keep the dilute dye solution in a dark bottle in the refrigerator. This solution should keep for at least three weeks.

**BUFFERED SODIUM ACETATE SOLUTION:**

Dissolve 4.53g of crystalline sodium acetate ( $\text{NaC}_2\text{H}_3\text{O}_2 \cdot 3\text{H}_2\text{O}$ ) in distilled water. Dilute to a volume of 100ml, and add 0.26ml of 0.5N acetic acid solution. Add a few drops of xylene as a preservative.

**INDOPHENOL-ACETATE SOLUTION:**

Mix equal volumes of the dye solution and the sodium acetate solution. Enough for several days may be made up and stored in the refrigerator. As a check on the quality of this solution, an excess of ascorbic acid may be added to a small portion, and complete decolorization should result.

**ASCORBIC ACID STANDARDS:**

**Stock Standard:** Dissolve 50mg of crystalline ascorbic acid in 50ml of 5% metaphosphoric acid solution and dilute in a volumetric flask to 100ml with distilled water. Mix and store in the refrigerator. This stock standard

contains 0.5mg ascorbic acid per ml in 2.5% metaphosphoric acid. It may be checked by titrating a 5ml portion plus a drop of starch solution to a faint blue color with 0.01N iodine solution. 1.0mg of ascorbic acid is equivalent to 1.14ml of 0.01N iodine solution.

**Dilute Standard:** Dilute 1.0ml of the stock standard with 50ml of 5% metaphosphoric acid, and distilled water to a total volume of 100ml. This solution contains 0.02mg of ascorbic acid in 4.0ml, and corresponds to a 2mg percent plasma ascorbic acid when used as described above.

As previously stated, the ascorbic acid standard is only needed to standardize the dye solutions which are made from a given lot of dye. Once this has been done, no further standard solutions are necessary.

## CALCIUM

### THE DETERMINATION OF CALCIUM IN BLOOD SERUM

Method of Roe and Kahn, J. Biol. Chem., 81, 1, (1929)

**FILTER:** Use Filter 66 (Red)

#### PROCEDURE:

##### UNKNOWN:

Add 1 volume of serum to 4 volumes of 10% trichloroacetic acid in a small flask and shake well. Pour onto a dry calcium-free filter paper (Whatman No. 42 or its equivalent) and collect the filtrate in a dry flask.

Place 5.0ml of the filtrate in a graduated 15ml conical centrifuge tube and add 1.0ml of 25% sodium hydroxide solution. Mix by lateral shaking and let stand 5 minutes. Add 1.0ml 5.0% trisodium phosphate, mix by lateral shaking and set aside for 1 hour. At the end of this time, centrifuge for 2 minutes and pour off the supernatant fluid, allowing the tube to drain in an inverted position for 2 minutes. Wipe the mouth of the tube dry with a clean cloth. Wash the precipitate with 5ml of alkaline-alcoholic wash reagent, delivered from a pipette with a fine tip, blowing the first portion of wash fluid against the precipitate with enough force to break it up, and using the remainder of wash fluid to rinse down the sides of the centrifuge tube. If necessary use a stirring rod to break up the precipitate. Centrifuge for 2 minutes, pour off the supernatant fluid and allow the tube to drain as before. After draining, wipe the mouth of the tube dry, and add 2.0ml of molybdate reagent, to dissolve the precipitate and form phosphomolybdate from the phosphate present. After a complete solution of the precipitate, which may be hastened by shaking or stirring, dilute to 10.0ml with distilled water, and mix well.

Transfer a 5.0ml portion from the centrifuge tube to a colorimeter tube and add 0.4ml of aminonaphtholsulfonic reagent, and dilute to the 10.0ml mark. Mix, wait 10 minutes and then read in the Colorimeter against a distilled water 0.

##### BLANK:

Treat a 5.0ml portion of distilled water with 1ml of molybdate reagent and 0.4ml of aminonaphtholsulfonic acid reagent, and dilute to the 10.0ml mark. Mix, wait 10 minutes and then read in the Colorimeter against a distilled water 0.

##### STANDARD:

Treat a 5.0ml portion of the standard phosphate solution with 1 ml of molybdate reagent, add 0.4ml of aminonaphtholsulfonic acid reagent, dilute with water to the 10.0ml mark. Mix, wait 10 minutes and then read in the Colorimeter against a distilled water 0. Subtract the value of the blank to obtain the true reading of the standard.

#### CALCULATION:

##### AGAINST THE STANDARD:

$$\frac{\text{Ca equivalent of standard}}{\text{Reading of standard}} \times \text{reading of unknown} = \text{serum calcium content in mg percent}$$

For the calcium equivalent of the standard use the value corresponding to whichever of the two standards described below is used. Other standards may be used provided the proper value is substituted in the equation above. The proportionality is excellent for all values of serum calcium likely to be encountered.

**USING A CALIBRATION FACTOR:**

Reading of unknown x serum calcium factor = serum calcium content in mg percent.

The factor is obtained from the reading of a standard solution as described above:

$$\text{serum calcium factor} = \frac{\text{Ca equivalent of standard}}{\text{Reading of standard}}$$

The value of the factor should be determined in duplicate or triplicate and the average value taken. Once determined, the calibration factor is valid indefinitely within the limits of this type of calibration, as discussed above.

**REAGENTS:**

All reagents should be of the highest quality, and free from significant amounts of calcium. The blank determination described above will not correct for the presence of calcium in the reagents. The calcium content of the reagents is best determined by running a complete determination as described above on a solution containing a known amount of calcium (e.g. 0.05mg Ca in 5ml) in place of the serum filtrate, and comparing the amount of calcium recovered with the amount added. If the reagents contain calcium, subtract the calcium equivalent of the reagents from the apparent serum calcium as obtained above, to obtain the true serum calcium.

**10% TRICHLOROACETIC ACID, 25% SODIUM HYDROXIDE, 5% TRISODIUM PHOSPHATE SOLUTIONS:**

Prepare on the indicated basis from distilled water and high grade reagent chemicals.

**ALKALINE-ALCOHOLIC WAS REAGENT:**

To 10ml of amyl alcohol, add 58ml of ethyl alcohol and mix. Dilute to a volume of 100ml with distilled water. Add 2 drops of 1% phenolphthalein solution and then drop by drop add sufficient 5% NaOH solution to a distinct pink color ( a few drops should be sufficient).

**MOLYBDATE REAGENT:**

Dissolve 25g of highest purity ammonium molybdate in 200ml of distilled water, and pour into a 1 liter volumetric flask containing 500ml of 10N sulfuric acid. Dilute to the mark and mix.

**AMINONAPHTHOLSULFONIC ACID REAGENT:**

This is prepared as described under the inorganic phosphate method of Fiske and Subbarow.

**STANDARD PHOSPHATE SOLUTIONS:**

The stock phosphate solution described under the inorganic phosphate method of Fiske and Subbarow is a convenient standard for the calcium determination. Dilute 5.0ml of the stock solution (containing 0.1mg phosphorus per ml) to 100ml with distilled water and mix. Since 0.517mg of phosphorus are equivalent to 1 mg of calcium as determined by this method, 5.0ml of the dilute phosphate standard corresponds to a calcium content of 9.67 mg percent on the basis of a 1:5 dilution of serum, when the analysis is carried out as described above. If a standard equivalent to 10.0mg percent of serum calcium is desired, dilute 5.17ml of the stock phosphate solution to 100ml with distilled water and mix. 5.0ml of this solution corresponds to a serum calcium of 10.0mg percent.

## PHOSPHORUS

**THE DETERMINATION OF INORGANIC PHOSPHATE IN WHOLE BLOOD, PLASMA OR SERUM**

Method of Fiske and Subbarow, J. Biol. Chem., 66, 375, (1925)

**FILTER:** Use Filter 66 (Red)

**PROCEDURE:****UNKNOWN:**

To 9 volumes of 10% trichloroacetic acid solution in a small flask, add 1 volume of whole blood, plasma or serum. Stopper the flask and mix by shaking, then pour onto a small dry ashless filter paper and collect the filtrate in a dry vessel.



Place 5.0ml of the filtrate in a colorimeter tube and add 1.0ml of molybdate reagent. Mix by lateral shaking, then add 0.4ml of the aminonaphtholsulfonic acid reagent. Dilute with water to the 10.0ml mark and mix by inversion. Allow to stand 5 minutes, then read in the Colorimeter against a distilled water 0. Subtract the reading of the blank from the reading of the unknown to obtain the true reading of the unknown.

If there is any turbidity present, the tube may be centrifuged during the 5 minute period before reading in the Colorimeter. As Fiske and Subbarow point out, a second reading taken about 5 minutes after the first reading may serve to indicate the presence of any unusual conditions in the analysis, if the rate of color change on the part of the unknown during this time is compared with that of the standard.

**BLANK:**

To a 5.0ml portion of 10% trichloroacetic acid in a colorimeter tube add 1.0ml of molybdate reagent and 0.4ml of aminonaphtholsulfonic acid reagent. Dilute to 10.0ml with water and mix. Allow to stand for 5 minutes and read in the colorimeter against a distilled water 0.

**STANDARD:**

To 5.0ml of standard phosphate solution, containing 0.025mg P, add 1.0ml of molybdate reagent and 0.4ml of aminonaphtholsulfonic acid reagent. Dilute to 10.0ml with water and mix. Allow to stand for 5 minutes, against a distilled water 0. Subtract the value of the blank from the reading of the standard to obtain the true reading of the standard.

**CALCULATION:**

**AGAINST THE STANDARD:**

$\frac{5.0}{\text{Reading of standard}} = \text{mg percent inorganic phosphate (as phosphorus) in original material.}$

The standard corresponds to a 1:10 dilution of blood, plasma or serum containing 5.0mg percent inorganic phosphate as phosphorus. Another standard may be used if the proper value is substituted in the formula above. The proportionality is excellent up to at least 15mg percent.

**USING A CALIBRATION FACTOR:**

Reading of unknown x inorganic phosphate factor = mg percent inorganic phosphate (as phosphorus) in original material.

The factor is obtained from the reading of a standard solution as described above:

$\text{inorganic phosphate factor} = \frac{5.0}{\text{Reading of standard}}$

The value of the factor should be determined in duplicate or triplicate and the average value taken. Once determined, the calibration factor is valid indefinitely within the limits of this type of calibration, as discussed above.

**REAGENTS:**

**10% TRICHLOROACETIC ACID:**

Prepare this on the indicated basis from high grade c.p. trichloroacetic acid. The blank determination will correct for the small amount of color-yielding material which may be present.

**MOLYBDATE REAGENT:**

Dissolve 25g of highest purity ammonium molybdate in 100ml distilled water. Pour into a 1 liter volumetric flask containing 300ml of 10N sulfuric acid and dilute to the mark with distilled water. Mix.

**AMINONAPHTHOLSULFONIC ACID REAGENT**

This is prepared from the recrystallized compound. The procedure of recrystallization may be found in the original article of Fiske and Subbarow. Weigh out 0.5g of the purified aminonaphtholsulfonic acid and place in the glass-stoppered cylinder containing 195ml of 15% sodium bisulfite and 5ml of 20% sodium sulfite. Shake until dissolved. If necessary to bring the powder into solution, add a little more of the sodium sulfite solution, but avoid an excess. Keep in a brown bottle away from light. This solution should be prepared fresh every two weeks.

## STANDARD PHOSPHATE SOLUTIONS (METAL CAP)

**Stock Standard:** Dissolve 0.439g of pure dry monopotassium phosphate in distilled water and dilute to 1 liter. Add a small amount of chloroform as a preservative. This solution contains 0.1mg P in 1.0ml.

**Working Standard:** Place 5.0ml of the stock standard in a 100ml volumetric flask and dilute to the mark with 10% trichloroacetic acid solution. Mix well. This solution contains 0.025mg P in 5.0ml.

# SULFANILAMIDE

## THE DETERMINATION OF SULFANILAMIDE IN BLOOD

Method of Marshall, J. Biol. Chem., 122, 263 (1937)

Marshall and Litchfield, Science, 88, 85 (1938)

**FILTER:** Use Filter 54 (Green)

### PROCEDURE:

#### UNKNOWN:

Measure 0.5ml of oxalated blood into a small flask containing 15.5ml of saponin solution. After laking is complete (1 to 2 minutes), add, while shaking, 4.0ml of para-toluene sulfonic acid solution. Wait 5 minutes and filter through a dry filter, collecting the filtrate in a dry flask.

Transfer 5.0ml of the filtrate to a colorimeter tube, add 0.5ml of a freshly prepared 0.1% sodium nitrite solution, and mix well by lateral shaking. Let stand 3 minutes then add, while shaking, 0.5ml of phosphate buffer containing ammonium sulfamate. Wait 2 minutes, then add 2.5ml of alcoholic dimethyl-alpha-naphthylamine solution. Mix well and allow to stand 10 minutes. Read in the Colorimeter against a distilled water 0.

The above procedures represents a blood dilution of 1:40. It is possible to use still higher dilutions (e.g. 0.1ml of blood in a final volume of 10ml, a 1:100 dilution) provided the proper acidity is maintained in the blood filtrate. Also distilled water may be used for laking instead of the saponin solution, and trichloroacetic acid may be used as the protein precipitant. For details see the original articles.

This procedure determines free sulfanilamide. For total sulfanilamide (free and conjugated), place a 5.0ml portion of the blood filtrate in a colorimeter tube and heat in boiling water for 90 minutes. Cool, add water to the 5.0ml mark, and carry out the colorimetric procedure as described above. The final reading represents total sulfanilamide, expressed as sulfanilamide.

When the determination of sulfapyridine is indicated, the procedure is the same as that described above, with the final value in terms of sulfanilamide being multiplied by the factor 1.5 to give the sulfapyridine content in mg percent.

#### BLANK:

With well prepared reagents, the blank value of this determination is negligible. It may be checked by running the colorimetric procedure as described above on a 5.0ml portion of a 1:5 dilution of the para-toluene sulfonic acid solution with distilled water.

#### STANDARD:

A dilute standard corresponding to 8mg percent of sulfanilamide at 1:40 dilution of the blood is used. Treat a 5.0ml portion of this standard exactly as described above for the free sulfanilamide in the blood filtrate. Read the final colored solution in the Colorimeter against a distilled water 0.

#### CALCULATION:

##### AGAINST THE STANDARD:

$$\frac{8.0}{\text{Reading of standard}} = \text{mg percent sulfanilamide in original blood}$$

This calculation is based on a standard equivalent to 8mg percent at a 1:40 dilution of the blood. Other standards and other dilutions of blood may be used, provided the proper values are substituted in the equation above. The proportionality of color to concentration is excellent over a wide range.

**USING A CALIBRATION FACTOR:**

Reading of unknown x blood sulfanilamide factor = mg percent sulfanilamide in blood.

The factor is determined from the reading of a standard solution as described above:

$$\text{blood sulfanilamide factor} = \frac{8.0}{\text{Reading of standard}}$$

The value of the factor should be determined in duplicate or triplicate and the average value taken. Once determined, the calibration factor is valid indefinitely within the limits of this type of calibration, as discussed above.

**REAGENTS:****SAPONIN SOLUTION:**

Dissolve 0.5g of saponin in 1 liter of distilled water.

**PARA-TOLUENE SULFONIC ACID SOLUTION:**

Dissolve 20g of the pure material in distilled water and dilute to 100ml.

**0.1% SODIUM NITRITE SOLUTION:**

Prepare fresh each day on the indicated basis, from reagent quality sodium nitrite.

**PHOSPHATE BUFFER CONTAINING AMMONIUM SULFAMATE:**

Dissolve 13.8g of sodium dihydrogen phosphate monohydrate ( $\text{NaH}_2\text{PO}_4\cdot\text{H}_2\text{O}$ ) in water, add 0.5g of ammonium sulfamate, and dilute to 100ml with water.

**ALCOHOLIC DIMETHYL-ALPHA-NAPHTHYLAMINE SOLUTION:**

Dilute 1ml of dimethyl-alpha-naphthylamine solution with sufficient 95% ethyl alcohol to make 250ml of solution. Keep in a dark bottle.

**STANDARD SULFANILAMIDE SOLUTIONS:**

**Stock Standard:** Dissolve 200mg of crystalline sulfanilamide in hot water and dilute to 1 liter. This solution will keep for months in the refrigerator.

**Acidified Dilute Standard:** This corresponds to 8mg percent of sulfanilamide at 1:40 dilution. Add 1.0ml of the stock standard to 18ml of para-toluene sulfonic acid solution in a 100ml volumetric flask, and dilute to the mark with distilled water. This standard should keep for one week. Other standards may be prepared by suitable dilution of the stock standard, and it is important to keep the acidity of the standard comparable to that of the blood filtrate.

## HEMOGLOBIN (HEMATIN)

### THE DETERMINATION OF BLOOD HEMOGLOBIN BY THE ACID HEMATIN METHOD

**FILTER:** Use Filter 54 (Green)

**PROCEDURE:**

Place 5.0ml of N/10 hydrochloric acid in a colorimeter tube. Collect 0.02ml of blood in a pipette calibrated to contain that amount. Remove excess blood from the outside of the pipette and then deliver the blood into the hydrochloric acid solution in the colorimeter tube. Rinse out the pipette by repeatedly drawing up and blowing back portions of the liquid in the tube, mixing the contents of the tube well with the tip of the pipette at the same time. Remove the pipette and allow the tube and contents to stand at room temperature for at least 30 minutes to permit maximum color development. Read in the Colorimeter against a 0.1N hydrochloric acid 0.

**CALCULATION:**

Reading of unknown x hemoglobin factor = gram percent hemoglobin.

**DETERMINATION OF FACTOR:**

This is obtained from the scale reading for a blood with known hemoglobin content. Procure a sample of fresh blood and determine its hemoglobin content by oxygen capacity or carbon monoxide capacity, or by the Wong method (described elsewhere in this book). Run a colorimetric determination as described above on duplicate or triplicate 0.02ml portions of this blood. From the hemoglobin content and the scale readings (averaged) the calibration factor is determined as follows:

$$\frac{\text{Gram percent hemoglobin in blood}}{\text{Average scaler reading}} = \text{calibration factor}$$

**REAGENTS:****N/10 HYDROCHLORIC ACID SOLUTION:**

This need not be exactly prepared, and may be made by diluting 1ml of concentrated hydrochloric acid to 100ml with distilled water.

**Note:** a calibrated liquid Acid Hematin Standard is available. This standard solution tube of known hemoglobin content is calibrated in grams per 100 ml of blood. It is the equivalent of 14.4g of hemoglobin per 100ml of blood in a 1:250 dilution as acid hematin.

## HEMOGLOBIN (OXYHEMOGLOBIN)

### THE DETERMINATION OF OXYHEMOGLOBIN IN BLOOD

**FILTER:** Use Filter 54 (Green)

**PROCEDURE:**

Place 5.0ml of dilute ammonia solution in a colorimeter tube. Collect 0.02ml of blood in a pipette calibrated to contain that amount. Remove excess blood from the outside of the pipette, place the tip of the pipette below the surface of the dilute ammonia solution and gently blow the contents of the pipette into the solution in the colorimeter tube. Rinse out the pipette by repeatedly drawing up and blowing back portions of the liquid in the tube, mixing well with the tip of the pipette at the same time. Remove the pipette and allow the solution to stand for a few minutes. Read in the Photoelectric Colorimeter against a distilled water 0.

**CALCULATION:**

Reading of unknown x oxyhemoglobin factor = gram percent hemoglobin

**DETERMINATION OF FACTOR:**

Procure a sample of fresh blood and determine oxyhemoglobin content by either the oxygen capacity or the carbon monoxide capacity method. Run a colorimetric determination on duplicate or triplicate 0.02 portions of this blood as described above. From the known hemoglobin content of the blood, the factor may be determined as follows:

$$\frac{\text{Gram percent hemoglobin}}{\text{Reading of standard}} = \text{oxyhemoglobin factor}$$

**REAGENTS:****DILUTE AMMONIA SOLUTION (0.4%):**

Dilute 4 ml of concentrated ammonium hydroxide solution to 1 liter with distilled water.

## IRON AND HEMOGLOBIN

### THE DETERMINATION OF IRON AND HEMOGLOBIN IN BLOOD

Method of Wong, J. Biol. Chem., 77, 409 (1928)

**FILTER:** Use Filter 54 (Green)

**PROCEDURE:**

**UNKNOWN:**

In a 50ml volumetric flask:

0.5ml blood (must be oxalated)

2.0ml concentrated sulfuric acid

Mix by whirling for at least 5 minutes. Add 2.0ml of saturated potassium persulfate solution and mix again for a few minutes. Dilute with distilled water to about half volume. Add 2.0ml of 10% sodium tungstate, mix by whirling and cool to room temperature under the cold water tap. Make up to the mark with distilled water, mix well by repeated inversion and pour onto a dry filter.

Transfer 10.0ml of the clear colorless filtrate to a colorimeter tube and add 0.5ml of saturated potassium persulfate solution followed by 2.0ml of 3N potassium thiocyanate solution. Mix by inversion and read in the Colorimeter within 30 minutes, against a blank tube set at 0.

**BLANK:**

In a 50ml volumetric flask containing about 25ml of distilled water:

2.0ml concentrated sulfuric acid

2.0ml saturated potassium persulfate solution.

Cool under the tap to room temperature and make up to the mark with distilled water. Mix well. Transfer a 10.0ml portion to a colorimeter tube, and develop the color exactly as described above for the unknown filtrate.

Place in the Colorimeter and set the instrument to its 0 reading.

**STANDARD:**

In a 50ml volumetric flask containing about 25ml of distilled water:

2.5ml of standard iron solution containing 0.1mg of ferric iron per ml (An equivalent quantity of any other standard iron solution available may be used).

2.0ml of concentrated sulfuric acid

2.0ml of saturated potassium persulfate solution

Cool under the tap to room temperature and make up to the mark with distilled water. Mix well. Transfer a 10.0ml portion of this solution to a colorimeter tube, and develop the color exactly as described for the unknown filtrate above. Read in the Colorimeter against the blank set at 0.

**CALCULATION:**

**AGAINST THE STANDARD:**

Since the standard iron solution is the equivalent of 50mg percent blood iron:

$$\frac{50}{\text{Standard reading}} \times \text{unknown reading} = \text{mg percent iron in original blood}$$

For direct reading as hemoglobin: 50mg percent iron corresponds to 14.9g percent hemoglobin. Therefore:

$$\frac{14.9}{\text{Standard reading}} \times \text{unknown reading} = \text{g percent hemoglobin in original blood}$$

**USING A CALIBRATION FACTOR:**

Unknown reading x Wong iron factor = mg percent iron in original blood

For direct reading as hemoglobin:

Unknown reading x Wong hemoglobin factor = g percent hemoglobin

The factor is obtained from the reading of the standard iron solution as described above:

$$\text{Wong iron factor} = \frac{50}{\text{Standard reading}}$$

$$\text{Wong hemoglobin factor} = \frac{14.9}{\text{Standard reading}}$$

The calibration factor should be determined in duplicate or triplicate and the average value taken. Once determined, the calibration factor is valid indefinitely within the limits of this type of calibration, as discussed above.

#### **REAGENTS:**

All reagents used should be of the highest quality and as free as possible from iron. This is particularly true of the sodium tungstate solution, since the blank reading as described above will correct for the presence of iron in all the reagents except the sodium tungstate.

#### **SATURATED POTASSIUM PERSULFATE SOLUTION:**

Shake 7g of potassium persulfate in a glass bottle containing 100ml of distilled water. Use the clean supernatant fluid, leaving the undissolved material in the bottle.

#### **10% SODIUM TUNGSTATE:**

Dissolve 100g of high grade sodium tungstate in sufficient distilled water to make 1 liter of solution.

#### **3N POTASSIUM THIOCYANATE SOLUTION:**

Dissolve 146g of highest purity potassium thiocyanate in sufficient distilled water to make 500ml of solution. Filter if necessary. Add 20ml of purest acetone if desired, to improve the keeping quality.

#### **STANDARD IRON SOLUTION:**

Dissolve 0.702g of reagent grade crystalline  $\text{Fe SO}_4 (\text{NH}_4)_2 \text{SO}_4 \cdot 6\text{H}_2\text{O}$  (Ferrous ammonium sulfate) in 100ml of distilled water. Add 5ml of concentrated  $\text{H}_2\text{SO}_4$ , warm slightly and add concentrated  $\text{KMNO}_4$  solution drop by drop until one drop produces a permanent color. Transfer to a 1 liter volumetric flask and dilute to 1 liter with distilled water. This solution contains 0.1mg of ferric iron per ml.

## **IRON**

### **THE DETERMINATION OF SERUM IRON**

Method of Schales, J. Biol. Chem., 135, 37-42, (1940)

**FILTER:** Use Filter 50 (Blue-green)

#### **PROCEDURE:**

##### **UNKNOWN:**

To 2 volumes of blood serum add 1 volume of 0.3N hydrochloric acid, and let stand for one hour. Then add 1 volume of 20% trichloroacetic acid solution, let stand for about ten minutes and centrifuge, or filter through an iron-free filter. Transfer 4.0ml of the filtrate to a Colorimeter tube, add 0.2ml of a 50% ammonium acetate solution, then 0.3ml of a 1% hydroquinone solution, and 1.0ml of a 0.1% o-phenanthroline solution. Let stand until the pink color is fully developed (about 20 minutes) and read in the Colorimeter against the blank tube set at 0. The color is stable.

##### **BLANK:**

Place 2.0ml of distilled water, plus 1.0ml of 0.3N HCl and 1.0ml of 20% trichloroacetic acid in a colorimeter tube. Add the ammonium acetate, hydroquinone, and o-phenanthroline exactly as described for the unknown, and read after 20 minutes or more in the Colorimeter. Set the instrument to its zero reading against the blank. This blank corrects for the unavoidable but very small iron content of the reagents, and should not read above 10 to 15 against distilled water, if C.P. reagents are used.

##### **STANDARD**

Place 2.0ml of standard iron solution, plus 1.0ml of 0.3N HCl, and 1.0ml of 20% trichloroacetic acid in a colorimeter tube. Add the ammonium acetate, hydroquinone, and o-phenanthroline solutions exactly as described above. Read in the Colorimeter against the blank tube set at 0, following the time conditions specified for the unknown.

## CALCULATION:

### AGAINST THE STANDARD:

$$\frac{200}{\text{Reading of standard}} \times \text{reading of unknown} = \text{micrograms percent iron in serum}$$

The standard is the equivalent of a serum containing 200 micrograms percent of iron. Other standards may be used if the proper value is substituted in the formula above. The proportionality is excellent for all values up to 1000 micrograms percent.

### USING A CALIBRATION FACTOR:

$$\text{Reading of unknown} \times \text{serum iron factor} = \text{micrograms percent iron in serum}$$

The value of the factor is obtained from the reading of a standard solution as described above:

$$\text{serum iron factor} = \frac{200}{\text{Reading of standard}}$$

The value of the factor should be determined in duplicate or triplicate and the average value taken. Once determined, the calibration factor is valid indefinitely within the limits of this type of calibration, as discussed above.

## REAGENTS:

### 0.3N HYDROCHLORIC ACID:

Dilute 30ml of concentrated hydrochloric acid to 1 liter with distilled water.

### 20% TRICHLOROACETIC ACID, 50% AMMONIUM ACETATE, 1% HYDROQUINONE, 0.1% O-PHENANTHROLIN:

Prepare on the indicated basis, from distilled water and high grade reagent chemicals.

### STANDARD IRON SOLUTION:

**Stock solution:** The standard iron solution is prepared from ferrous ammonium sulfate - Fe (SO<sub>4</sub>)(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>·6H<sub>2</sub>O. A stock solution containing 0.01 mg Fe per ml is made by dissolving 70.2mg of the salt in 1000ml of distilled water containing a few drops of dilute sulfuric acid.

**Working solution:** The working standard, containing 2 micrograms per ml (equivalent to 200 micrograms percent) is prepared by diluting 20ml of the stock standard to 100ml with distilled water.

**Note:** This method for serum iron can be used for any kind of iron - even after the digestion of the material with sulfuric acid and hydrogen peroxide (This is necessary only if the iron is in an organic complex, as in hemoglobin). The formation of the color is considerably delayed if oxalate is present, and if one wished to do determinations on blood (e.g. determination of pseudo-hemoglobins, the easily split off iron of Barkan and Schales) one should use heparin to prevent clotting, or use defibrinated blood, but not oxalated blood.

## ICTERUS INDEX

### DETERMINATION OF ICTERUS INDEX

Method of Summerson

**FILTER:** Use Filter 42 (Blue)

### PRINCIPLE:

The yellow color of serum is compared with that of standard potassium dichromate solution. The test is reported in terms of the relative color of serum and standard.

The Icterus Index of normal persons seldom exceeds 6, but may occasionally be as high as 9. Jaundice does not become visible clinically until the Icterus Index reaches 15 to 20.

**PROCEDURE:**

**UNKNOWN:**

Transfer 1ml of serum (be sure that there is no hemolysis) to a colorimeter tube and add 9ml of 5% Sodium Citrate solution. Mix several times by inversion and read in the Colorimeter against distilled water set at 0.

If the reading is above 400, transfer 1ml of the mixture to another colorimeter tube, dilute to 10ml with distilled water, and read in the Colorimeter.

**CALCULATION:**

Reading of unknown x Icterus Index Factor = Icterus Index

If the second dilution of 1:10 was made, then the formula is modified to:

Reading of unknown x 10 x factor = Icterus Index.

The factor is obtained from the reading of a standard solution having an equivalent Icterus Index of 10.

$$\frac{10}{\text{Reading of standard}} = \text{Icterus Index Factor}$$

The calibration factor should be determined in duplicate or triplicate and the average value taken. The calibration factor is constant up to readings of 400.

**REAGENTS:**

**STANDARD POTASSIUM DICHROMATE SOLUTION:**

**Stock solution:** A 1.00% stock solution is made by dissolving 10.000g of C.P.  $K_2Cr_2O_7$  in distilled water, adding 1 or 2 drops of sulfuric acid, and diluting to 1 liter with distilled water.

**Working solution:** The standard with an equivalent value of 10 is prepared by diluting 1ml of the stock solution to 100ml with distilled water.

**SODIUM CITRATE SOLUTION, 5%:**

Dissolve 50g of C.P. or U.S.P. Sodium Citrate in distilled water and dilute to 1 liter. If turbid, allow to stand overnight and decant.

## ALBUMIN AND GLOBULIN

### THE DETERMINATION OF SERUM ALBUMIN AND SERUM GLOBULIN; A/G RATIO

Method of Looney and Walsh, J. Biol. Chem., 130, 635, (1939)

**FILTER:** Use Filter 42 (Blue)

**PROCEDURE - GLOBULIN:**

**UNKNOWN:**

Dilute 1ml of blood serum to 10ml with 1% salt solution. Place 1ml of this dilute serum in a colorimeter tube, and add 2ml of 2% gum ghatti, followed by 2ml of saturated ammonium sulfate. Mix by inversion several times, wait 10 minutes and read in the Colorimeter against a distilled water 0.

**STANDARD:**

Treat 1ml of a standard globulin solution with gum ghatti and ammonium sulfate, exactly as described for the unknown. Wait 10 minutes and read in the Colorimeter against a distilled water 0.



## **CALCULATION:**

### **AGAINST THE STANDARD:**

$$\frac{\text{Globulin content of standard}}{\text{Reading of standard}} \times \text{reading of unknown} = \text{globulin content of unknown}$$

If the globulin content of the standard is expressed in mg percent, then the value for the unknown will be directly in mg percent.

### **USING A CALIBRATION FACTOR:**

Reading of unknown x globulin factor = concentration of globulin in unknown.

The value of the factor is determined from the reading of a standard globulin solution, as described above:

$$\text{globulin factor} = \frac{\text{Globulin content of standard}}{\text{Reading of standard}}$$

The value of the factor should be determined in duplicate or triplicate and the average value taken. Once determined, the calibration factor is valid indefinitely within the limits of this type of calibration, as discussed above.

## **REAGENTS:**

### **1% SALT SOLUTION:**

Prepare on the indicated basis from reagent quality sodium chloride and distilled water.

### **2% GUM GHATTI:**

Suspend 20g of soluble gum ghatti by means of a wire screen or cloth bag just below the surface of a liter of cold distilled water in a cylinder. Leave 18 hours or longer, remove the bag or screen and strain the fluid in the cylinder through a double layer of clean towel.

### **STANDARD GLOBULIN SOLUTION:**

Treat blood serum with an equal volume of saturated ammonium sulfate, and centrifuge off the precipitated globulin as completely as possible. Suspend the precipitate in distilled water and dialyze against running tap water until the contents of the dialyzing sack are free from ammonium salts (test a small portion of the filtered suspension with Nessler's solution).

When the ammonium salts have been removed, dilute the suspension with an equal volume of 2% sodium chloride solution. Determine the nitrogen content of the solution by the Kjeldahl Method. Multiply the percent nitrogen by 6.25 to obtain the percent globulin.

From this stock solution, suitable working standards may be prepared by simple dilution with 1% sodium chloride.

## **PROCEDURE - ALBUMIN: (TOTAL PROTEIN)**

The albumin concentration is obtained by taking the difference between the total protein value of the serum and the globulin content. The following directions are for the determination of total protein in serum.

### **UNKNOWN:**

Prepare a 1% solution of serum by diluting the first serum mixture again in the proportion of 1:10 by the addition of 1% salt solution. Place 2ml of this dilution in a colorimeter tube and add 0.5ml of 2% gum ghatti and 2.5ml of 5% sulfosalicylic acid. Read the resulting turbid solution in the Colorimeter against distilled water set at 0.

### **STANDARD:**

A standard serum, whose protein content is known, is diluted and treated exactly as the unknown with gum ghatti and sulfosalicylic acid. The reading is made in the Colorimeter against a distilled water 0.

## **CALCULATION:**

### **AGAINST THE STANDARD:**

$$\frac{\text{Protein content of standard}}{\text{Reading of standard}} \times \text{reading of unknown} = \text{protein content of unknown}$$

If the protein content of the standard is expressed in mg percent, the value for the unknown will be directly in mg percent protein.

**USING A CALIBRATION FACTOR:**

Reading of unknown x protein factor = concentration of protein in unknown.

The value of the factor is determined from the reading of a standard protein solution, as described above:

$$\text{protein factor} = \frac{\text{Protein content of standard}}{\text{Reading of standard}}$$

The value of the factor should be determined in duplicate or triplicate and the average value taken. Once determined, the calibration factor is valid indefinitely within the limits of this type of calibration, as discussed above.

**REAGENTS:**

**1% SALT SOLUTION, 2% GUM GHATTI:**

See determination of albumin.

**5% SULFOSALICYLIC ACID:**

Prepare on the indicated basis from good quality material and distilled water.

**STANDARD PROTEIN SOLUTION:**

The standard protein solution described in the procedure for the determination of protein in cerebrospinal fluid (see next section) may be used. The working standard is prepared by suitable dilution of the stock solution with 1% sodium chloride.

**Note:** It is possible to reduce the amount of serum used to 0.2ml diluting this amount to 2ml, and taking 0.5ml for the second dilution.

## PROTEIN IN CEREBROSPINAL FLUID

### THE DETERMINATION OF PROTEIN IN CEREBROSPINAL FLUID

**FILTER:** Use Filter 42 (Blue)

**PROCEDURE:**

**UNKNOWN:**

Place 1.0ml of the clear colorless unknown fluid in a colorimeter tube, add 4.0ml of 3% sulfosalicylic acid solution, and mix by gentle inversion. Allow to stand for 10 minutes. Read in the Colorimeter against a distilled water 0, mixing the contents of the tube by gentle inversion before making the reading if flocculation is evident. The reading may be made any time between 10 and 30 minutes after the addition of the sulfosalicylic acid.

**STANDARD:**

Treat 1.0ml of the standard protein solution with 4.0ml of 3% sulfosalicylic acid, as described above. Read in the Colorimeter between 10 and 30 minutes after the addition of the precipitating agent, against a distilled water 0.

**CALCULATION:**

**AGAINST THE STANDARD:**

$$\frac{\text{Protein content of standard}}{\text{Reading of standard}} \times \text{reading of unknown} = \text{protein content of unknown}$$

If the protein content of the standard is expressed in mg percent the value for the unknown will be directly in mg percent protein. The proportionality is adequate for all concentrations of protein which may be read without too much flocculation taking place. If this occurs, the determination should be repeated on a diluted portion of the sample.

**USING A CALIBRATION FACTOR:**

Reading of the unknown x turbidimetric protein factor = concentration of protein in unknown.

The value of the factor is determined from the reading of a standard protein solution, as described above:

$$\text{turbidimetric protein factor} = \frac{\text{Protein content of standard}}{\text{Reading of standard}}$$

The value of the factor should be determined in duplicate or triplicate and the average value taken. Once determined, the calibration factor is valid indefinitely within the limits of this type of calibration, as discussed above.

**REAGENTS:**

**3% SULFOSALICYLIC ACID**

Prepare on the indicated basis from good quality material and distilled water.

**STANDARD PROTEIN SOLUTION:**

**Stock Standard:** Dilute a pooled lot of normal human serum to 10 times its volume with 15% sodium chloride solution and filter. The total nitrogen of the stock solution is determined by the Kjeldahl method, and from the value for total nitrogen the non-protein nitrogen value is subtracted, to give the protein nitrogen content of the stock standard. The non-protein nitrogen may be determined by the Koch and McMeekin described in a previous section, or an average value of 30mg percent for the undiluted serum may be taken.

From the protein nitrogen content of the stock standard, the protein content is obtained by multiplying by the factor 6.25. The value for the usual sock standard should be around 0.7g percent. This standard keeps well if stored in the refrigerator.

**Working Standard:** The dilute working standard is prepared from the stock standard by suitable dilution. If the stock solution contains 0.7g percent of protein, a 1:20 dilution would give a standard equivalent to 35mg percent protein.

## PHENOLSULFONEPHTHALEIN IN URINE

### THE DETERMINATION OF PHENOLSULFONEPHTHALEIN IN URINE

(Renal function test of Rowntree and Geraghty)

**FILTER:** Use Filter 54 (Green)

**PROCEDURE:**

To each hour sample of urine add 10% sodium hydroxide solution, drop by drop, until the maximum color is obtained. Dilute the solution to 1 liter with distilled water and mix.

If insufficient alkali is present, the diluted solution will not have its maximum color. This may be avoided by routinely adding 1 or 2 drops of alkali to the (approximately) 10ml portion of the sample in the colorimeter tube before reading.

The concentration of dye in the sample is obtained from the reading of the sample and the reading of a standard containing a known amount of dye. A convenient standard for this purpose contains 3mg of phenolsulfonephthalein in 1 liter, in the presence of a small amount of alkali to bring out the maximum color. Under these conditions:

$$\text{mg dye in sample} = \frac{3.0}{\text{Reading of standard}} \times \text{reading of sample}$$

The percent excretion in the sample is obtained as follows:

$$\text{percent excretion} = \frac{\text{mg dye in sample}}{\text{mg dye injected}}$$

**USING A CALIBRATION FACTOR:**

With the standard described above:

$$\text{PSP factor} = \frac{3.0}{\text{Reading of standard}}$$

$$\text{percent excretion} = \frac{\text{Reading of sample} \times \text{PSP factor}}{\text{mg dye injected}}$$

# ANDROSTERONE

## THE DETERMINATION OF ANDROSTERONE

Method of Sachs and Kurzrok, unpublished

**FILTER:** Use Filter 45 (Green)

### PROCEDURE:

#### UNKNOWN:

**Combined hydrolysis and extraction:** A 48 hour sample of urine is collected, and the volume measured. One liter of the urine is placed in a flask fitted with a ground-in reflux condenser. Add 150ml of concentrated hydrochloric acid and 125ml of carbon tetrachloride and boil on a water bath for two hours. As soon as it has cooled sufficiently, the lower layer of  $\text{CCl}_4$  is removed by means of separation in a separatory funnel. This  $\text{CCl}_4$  layer is transferred to another separatory funnel, and any urine washed back into the flask. The extraction is repeated in the same way, for 2 hour periods, twice more. The  $\text{CCl}_4$  is distilled off from the united extracts, and the residue is taken up in 100ml of benzene.

**Fractionation:** The benzene solution is freed from acid by washing twice with 25ml portions of saturated sodium bicarbonate solution, freed from phenols by extracting five times with 20ml portions of 2N sodium hydroxide, and washed with water. Should phenolic preservatives have been used, the extraction with NaOH must be repeated after acidification until a portion of the extract produces no cloudiness.

The benzene solution, which contains the androsterone, is evaporated to dryness and the residue extracted four or five times with 5 to 10ml portions of redistilled ether. The extract is transferred to a beaker and stirred with 0.5g of decolorizing charcoal. It is then filtered through a Whatman No. 40 dry filter paper, and the filtrate collected in a distillation flask. The remaining charcoal and the separatory funnel are washed with about 70ml of ethyl ether and the filtrate combined with the first filtrate. Then the ether is distilled off on a hot water bath. After cooling slowly to room temperature, the residue in the distillation flask is carefully dissolved in 20ml of 95% alcohol.

Place 4.0ml of the alcoholic solution in a colorimeter tube, and dilute with 95% alcohol to the 10ml mark. Then add 2.0ml of 15% aqueous potassium hydroxide, and 2.0ml of 2% m-dinitrobenzene. Mix by inversion and allow to stand in the dark for 90 minutes. Read in the Colorimeter against the blank tube set at 0.

#### BLANK:

Place 10.0ml of 95% alcohol in a colorimeter tube, and add the potassium hydroxide and m-dinitrobenzene exactly as described for the unknown. Mix by inversion and allow to stand in the dark for 90 minutes. Place in the Colorimeter and set the instrument to its 0 reading against the blank.

#### STANDARD:

Place 2.0ml of standard androsterone solution (containing 0.4mg in 2ml) plus 8.0ml of 95% alcohol in a colorimeter tube, and continue the colorimetric procedure exactly as described above. Read in the Colorimeter against the blank tube set at 0, following the time conditions specified for the unknown.

#### CALCULATION:

$$\frac{0.4}{\text{Reading of standard}} \times \text{reading of unknown} = \text{mg androsterone in 1 liter of urine}$$

Mg androsterone in 1 liter x total volume = total excretion in 48 hours

$$\frac{\text{Total excretion}}{2} = \text{mg androsterone in 24 hour sample}$$

A calibration factor cannot be used for this determination since the color produced is affected to a very large extent by conditions such as temperature, reagents, etc. For precise results, a blank must be run, and the unknown compare each time against a standard, as described in the procedure.

**REAGENTS:****SATURATED SODIUM BICARBONATE SOLUTION:**

Shake about 20g of NaHCO<sub>3</sub> in a glass stoppered bottle containing 100ml of distilled water. Use the clear supernatant fluid, leaving the undissolved material in the bottle.

**2N SODIUM HYDROXIDE:**

Dissolve 80.01g of NaOH in cold distilled water which has been previously boiled to expel CO<sub>2</sub>, and dilute to 1 liter with CO<sub>2</sub>-free water.

**AQUEOUS POTASSIUM HYDROXIDE:**

Dissolve 15g of KOH in sufficient distilled water to make 100ml of solution.

**2% META-DINITROBENZENE:**

Dissolve 2g of m-dinitrobenzene in sufficient 95% alcohol to make 100ml of solution.

**STANDARD ANDROSTERONE SOLUTION:**

**Stock solution:** Dissolve 0.100g of crystalline androsterone in sufficient 95% alcohol to make 100ml of solution. This solution contains 1mg of androsterone in 1ml.

**Working Standard:** Dilute the stock solution 1:5 with alcohol so that 2ml = 0.4ml androsterone.

## TABLE OF NORMAL VALUES

Sugar (Folin)	90 - 120mg/100ml	
Sugar (Benedict)	70 - 100mg/100ml	
Sugar (Folin-Malmros)	75 - 105mg/100ml	
N.P.N.	25 - 35mg/100ml	
Uric Acid	2 - 3.5mg/100ml	
Urea	10 - 15mg/100ml	
Creatinine	1 - 2mg/100ml	
Bilirubin	0.2 - 0.6mg/100ml	
Cholesterol	160 - 240mg//100ml	
Vitamin C	0.8 - 2.4mg/100ml	
Calcium	9 - 11.5mg/100ml	or 4.5 - 5.6 meq/liter
Inorganic Phosphate	3.0 - 4.7mg/100ml in adults	5mg/100ml in children
Sulfanilamide	0 - 20mg%	
Hemoglobin	13.5 - 15.0 g% females	14.5 - 16.0 g% males
Iron	46 - 55 mg/100ml	
Serum Iron	0.05 - 0.25mg/100ml	
Icterus Index	4 - 6	
Albumin (Serum)	4.6 - 6.7 gm/100ml	
Globulin (Serum)	1.2 - 3.2 gm/100ml	
Protein (Spinal Fluid)	20 - 40 mg%	
P.S.P.	40 - 60% 1st hour	20 - 25% 2nd hour
Acid Phosphatase	0 - 1.1 units	
Alkaline Phosphatase	1.5 - 4.0 units	
Chloride	570 - 620 mg/100ml	
	or 98 - 106 meq/liter	

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## % TRANSMITTANCE INFORMATION

The Klett-Summerson Colorimeter scale is graduated in units proportional to the optical density. The actual numerical values represent the optical density divided by two, with the decimal point omitted. For example, a scale reading of 250 corresponds to an optical density of 0.500. The formula relating scale reading and optical density is as follows:

$$(1000/2) \times D = R$$

where D is the density, and R is the reading.

It is sometimes desired to obtain scale reading in terms of percent transmittance (less correctly called percent transmission). For this purpose, the accompanying table may be used. From this table the optical density and the percent transmittance corresponding to any Klett-Summerson scale reading may be readily obtained.

It is also possible to calculate the percent transmittance value corresponding to any Klett-Summerson scale reading. This is done as follows:

1. Obtain the optical density, by multiplying the scale reading by 0.002.
2. Subtract the optical density value from 2.000. The result is the logarithm of the percent transmittance. From logarithm tables, obtain the value of the percent transmittance.

Example    Scale reading = 150  
              Optical density =  $150 \times 0.002 = 0.300$   
              log % transmittance =  $2.000 - 0.300 = 1.7$   
              % transmittance = 50.2%

The accompanying table affords a convenient way of obtaining optical density values from scale readings.



## % TRANSMITTANCE CHART

READING	OPTICAL DENSITY	% TRANSMITTANCE	READING	OPTICAL DENSITY	% TRANSMITTANCE	READING	OPTICAL DENSITY	% TRANSMITTANCE
0.0	0.000	100	90.5	0.181	66	247.5	0.495	32
2.0	0.004	99	93.5	0.187	65	254.5	0.509	31
4.5	0.009	98	97.0	0.194	64	261.5	0.523	30
6.5	0.013	97	100.5	0.201	63	269.0	0.538	29
9.0	0.018	96	104.0	0.208	62	276.0	0.552	28
11.0	0.022	95	107.5	0.215	61	284.5	0.569	27
13.5	0.027	94	111.0	0.222	60	292.5	0.585	26
16.0	0.032	93	114.5	0.229	59	301.0	0.602	25
18.0	0.036	92	118.5	0.237	58	310.0	0.620	24
20.5	0.041	91	122.0	0.244	57	319.0	0.638	23
23.0	0.046	90	126.0	0.252	56	329.0	0.658	22
25.5	0.051	89	130.0	0.260	55	339.0	0.678	21
28.0	0.056	88	134.0	0.268	54	349.5	0.699	20
30.5	0.061	87	138.0	0.276	53	360.5	0.721	19
33.0	0.066	86	142.0	0.284	52	372.5	0.745	18
35.5	0.071	85	146.0	0.292	51	385.0	0.770	17
38.0	0.076	84	150.5	0.301	50	398.0	0.796	16
40.5	0.081	83	155.0	0.310	49	412.0	0.824	15
43.0	0.086	82	159.5	0.319	48	425.0	0.850	14
46.0	0.092	81	164.0	0.328	47	443.0	0.886	13
48.5	0.097	80	168.5	0.337	46	460.5	0.921	12
51.0	0.102	79	173.5	0.347	45	479.5	0.959	11
54.0	0.108	78	178.5	0.357	44	500.0	1.000	10
57.0	0.114	77	183.5	0.367	43	523.0	1.046	9
59.5	0.119	76	188.5	0.377	42	548.5	1.097	8
62.5	0.125	75	193.5	0.387	41	577.5	1.155	7
65.5	0.131	74	199.0	0.398	40	611.0	1.222	6
68.5	0.137	73	204.5	0.409	39	650.5	1.301	5
71.5	0.143	72	210.0	0.420	38	699.0	1.398	5
74.5	0.149	71	216.0	0.432	37	761.5	1.523	3
77.5	0.155	70	222.0	0.444	36	849.5	1.699	2
80.5	0.161	69	228.0	0.456	35	1000.0	2.000	1
84.0	0.168	68	234.5	0.469	34			
87.0	0.174	67	241.0	0.482	33			



WAYNE, NJ 07470 USA

## Operating Instructions

# Klett Photoelectric Colorimeter



Industrial Model

Catalog No. T37013-0000  
Catalog No. T37013-0001



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# PRINCIPALS OF OPERATION

This is a Photoelectric Colorimeter using specific light filters of the visible range (380nm-740nm). The scale readings are directly proportional to the concentration in accordance with Beers law.

Two matched photocells of the "blocking layer" type, in a fully compensated and carefully balanced electrical circuit, form the basis for current measurement. The galvanometer is of the suspension wire type.

## SPECIFICATIONS

T37013-0000	Colorimeter	Glass Cell Model 900-3	110V	100W
T37013-0001	Colorimeter	Glass Cell Model 900-3	220V	100W

## OUTSIDE DIMENSIONS

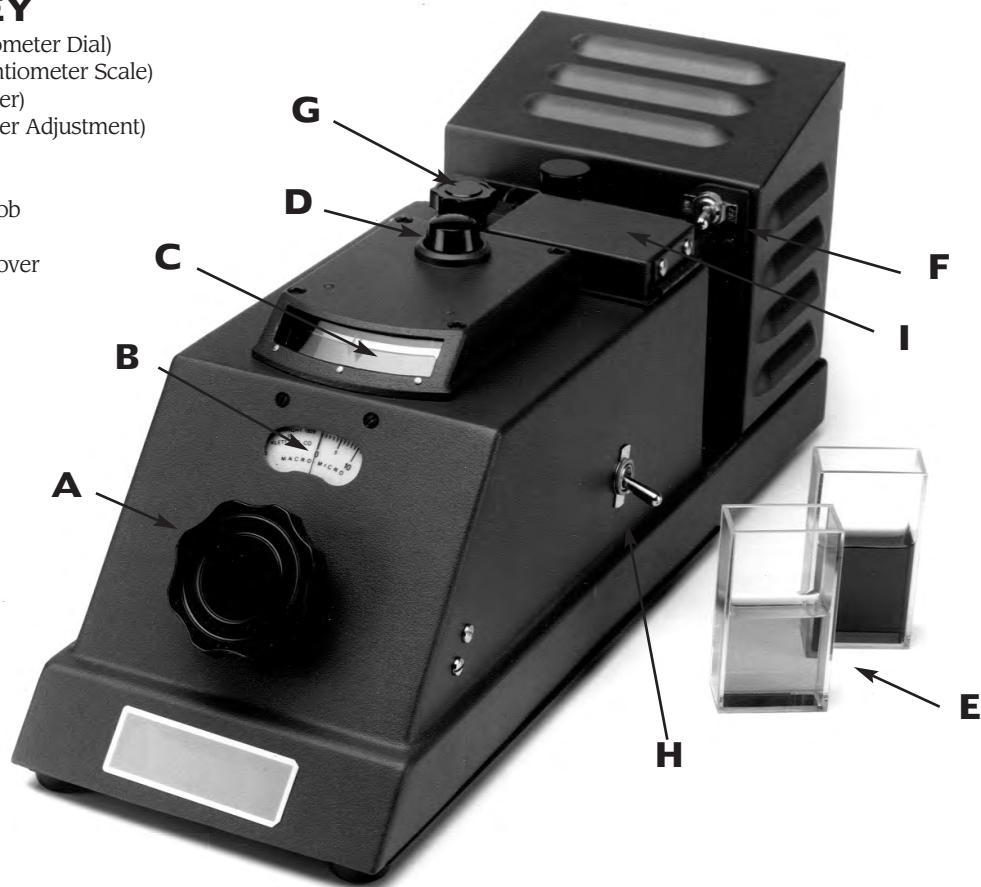
Length:	17.5 in. (44.5cm)	Height:	7.5 in (19cm)
Width:	6 in (15cm)	Weight:	12.5 lbs. (5.7 kg)

## SHIPPING SPECIFICATIONS

Size:	20 x 16 x 13 <sup>7</sup> / <sub>8</sub> in. (50.8 x 40.6 x 35.2 cm)	Weight:	21 lbs. (9.5 kg)
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## KEY

- A** Scale Knob (Potentiometer Dial)
- B** Scale Reading (Potentiometer Scale)
- C** Pointer (Galvanometer)
- D** Galvanometer (Pointer Adjustment)
- E** Solution Cell
- F** Light Switch
- G** Zero Adjustment Knob
- H** Short-Circuit Switch
- I** Cell Compartment Cover



**FIGURE 1**

# CONTROLS

## THE ZERO ADJUSTMENT KNOB

The small knob located at the top of the Colorimeter, to the left of the solution cell. This knob enables the pointer to be brought to its zero position at the line of the pointer scale when the Colorimeter lamp is on.

## GALVANOMETER ADJUSTMENT KNOB (POINTER)

The zero position for the galvanometer pointer is when the tip of the pointer is exactly on the single line on the pointer scale. The pointer should be in this position when the instrument is not in operation and the lamp is turned off.

All colorimetric measurements are based on the pointer being at its zero position when distilled water or the reagent blank solution is in place and the scale reads 0. The accuracy of the instrument depends to a large extent on how carefully the zero adjustment is made.

**Note:** During the use of the instrument the pointer may occasionally swing to the extreme end of the scale with considerable force. This does no harm provided the pointer is not left for any length of time in that position, but is brought back to the center of the scale as quickly as possible.

## GALVANOMETER SWITCH

To protect the pointer mechanism from damage during shipping, there is a toggle switch (H). This switch should be set to "OFF" **only** when moving or shipping the Colorimeter. For every day use, this switch should be set in the "ON" position, otherwise the pointer will not move when the lamp is turned on.

## LAMP SWITCH

The lamp switch, located on the front of the lamp housing, is designed so that the filters cannot be removed from the Colorimeter without switching the lamp off. This is to minimize the possibility of strong unfiltered light striking the photoelectric cells.

## THE COLORIMETER LAMP

This is a standard 100 watt double filament bulb and operates from the standard electrical AC or DC line. The lamp bulb is not operated at its full rating in order to prolong the life of the lamp.

The lamp should be switched off when the instrument is not in use, but it will not cause any damage if it is left on for intermittent use throughout the day.

# KEY ELEMENTS

## LIGHT FILTERS

The light filter should be inserted in the filter holder with the round opening facing forward. To prevent damage to the photocells, the Colorimeter lamp should not be turned on unless there is a filter in place. For best results, insert this filter in the filter holder with the engraved number located in the lower right corner, facing you. Due to variation in the raw glass, filters from different batches may appear to be a different color. This will not affect the transmissivity of the filter.

## CHOICE OF PROPER FILTER

For best results it is important that the proper light filter is used. It has been found that the three most popular filters will cover most ordinary colorimetric requirements. There are also other filters for your needs.

The proper filter is usually specified in the directions for the procedure. Ordinarily the filter selected is the one with a spectral transmission opposite to that of the solution being measured, i.e. the filter which transmits the most light over the range where the solution absorbs the most light. In this way maximum sensitivity is usually obtained. Furthermore, it is frequently possible by use of proper filters to read two colors, both present in the same solution, independently of each other, or to specifically measure one colored compound in the presence of an extraneous color.

When changing from one filter to another, the change in the color of light striking the photoelectric cells may disturb the balance between them temporarily, so the instrument should run for about 5 to 10 minutes with the new filter in place before making measurements.

It is necessary to reset the zero with distilled water when a new filter is in place in the Colorimeter, since the cells in balance for one filter are not necessarily in balance for another.



CATALOG NUMBER	FILTER NUMBER	COLOR	APPROXIMATE SPECTRAL RANGE (NANOMETERS)	COLOR OF SOLUTION FOR WHICH FILTER IS USED
T37014-0042	KS-42	BLUE	400-450	RED, ORANGE, YELLOW, GREEN, BLUE, TURBID
T37014-0054	KS-54	GREEN	520-580	RED, YELLOW, PURPLE, ORANGE, BLUE
T37014-0066	KS-66	RED	640-700	BLUE, GREEN, YELLOW

CATALOG NUMBER	FILTERS	TRANSMISSION LIMITS IN NANOMETERS																																						
		380	390	400	410	420	430	440	450	460	470	480	490	500	510	520	530	540	550	560	570	580	590	600	610	620	630	640	650	660	670	680	690	700	710	720	730	740		
T37014-0040	KS-40	█																																						
T37014-0042	KS-42			█	█	█	█	█																																
T37014-0044	KS-44				█	█	█	█	█	█																														
T37014-0047	KS-47										█	█	█	█	█																									
T37014-0050	KS-50												█	█	█	█	█																							
T37014-0052	KS-52															█	█	█	█	█																				
T37014-0054	KS-54																																							
T37014-0055	KS-55																																							
T37014-0056	KS-56																																							
T37014-0059	KS-59																																							
T37014-0060	KS-60																																							
T37014-0062	KS-62																																							
T37014-0064	KS-64																																							
T37014-0066	KS-66																																							
T37014-0069	KS-69																																							
T37014-0070	KS6225																																							

Filters should not be left in machine with lamp on.

The spectral transmission curve of a typical filter (no. 52 is selected) is illustrated. The spectral specificity of this filter is indicated by the fact that 85% of all the light transmitted has a wavelength between 485 and 550 nanometers.

**THE SOLUTION CELLS: CAT. NO. T37013-0010 (20, 40MM) T37013-0020,(10MM)**

The solution cells are specially constructed using a resistant cement and therefore can be used with acids, alkalis and most organic solvents.

Measurements can be carried out at solution depths of 40mm., 20mm., 10mm., or 2.5 mm. The 2.5mm solution depth is obtained by the use of a special reduction plate (Cat. No. T37013-0030) in the 10mm cell. Before running the determination, decide on which solution cell to use. The choice depends on the solution used; the large cells are for very dilute solutions or suspensions, the smaller cells for dark solutions, etc.

The solution in the cell, should be at least 5cm high. Solutions should always be at room temperature before readings are made.

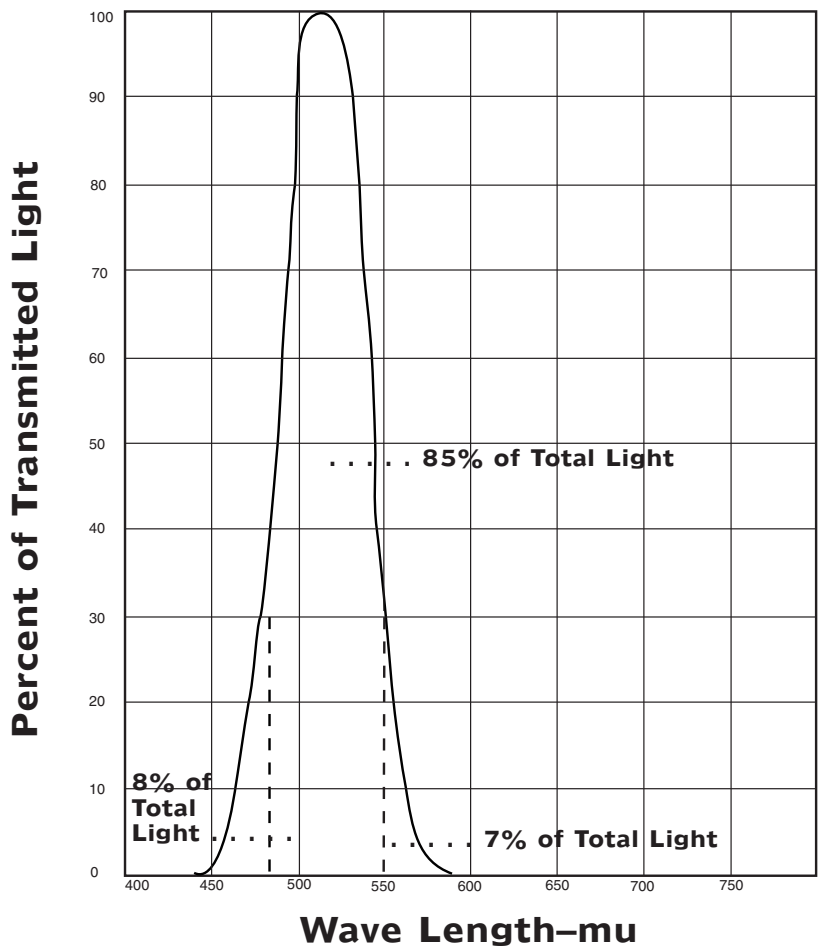
It should be noted that the zero setting for one cell and solution density is not necessarily the same when another cell and solution thickness are used. It is necessary to reset the zero whenever the solution thickness is changed. If desired, the standard Colorimeter test tube, or micro tube may be readily used in place of the rectangular solution cells, in conjunction with the special adapter (Catalog # 37013-0041).

**TEST TUBES: CAT. NO. T37012-0010 (UNGRADUATED) T37012-0020 (GRADUATED)**

The macro test tubes can be heated in boiling water in the event that the colorimetric procedure calls for it. These test tubes may also be used for centrifugation to remove turbidity (as described in TURBIDIMETRIC MEASURES below).

**MICRO TUBES: CAT. NO. 93701-2012 (FLAT BOTTOM, 2.5ML) DISC**

Micro tubes are used for those analytical procedures which require volumes as little as 2.5ml. These tubes are similar to



the regular tubes, but have flat bottoms. Care should be taken when adding hot solutions, or heating these tubes in boiling water, since the flat bottoms of the micro tubes are less resistant to heat shock than the rounded bottom macro tubes.

## USE OF COLORIMETER SCALE

### READING

The Klett-Summerson Photoelectric Colorimeter scale is specifically designed to enable the analyst to take full advantage of the validity of Beers Law for the colorimetric procedure being used.

Because of the design of the Colorimeter and the use of highly selective light filters, it has been found that Beers Law is valid for practically all of the common colorimetric procedures under the conditions of this use in the Colorimeter.

The scale is logarithmically spaced, not linearly spaced. Therefore, results are obtained by simple calculation from the scale reading, eliminating the need for calibration curves or semi-logarithmic paper.

For the majority of colorimetric procedures the reading will fall between 0 and 200 or 300. Readings above 500 should not be used as a basis for calculating results and solutions should be diluted accordingly.

### CALCULATION OF RESULTS USING A CALIBRATION FACTOR

The logarithmic scale makes calculations very easy:

1. The reading of the unknown (corrected for a blank) is directly proportional to its concentration.
2. For best results, run a standard solution along with the unknown. Since the readings for both the standard and the unknown are proportional to the concentration, the results are calculated by use of the following formula:
3. Factor x reading of unknown = concentration of unknown.
4. The value of the factor is obtained from the scale reading for a solution of known concentration.

5. Factor =  $\frac{\text{Concentration of standard}}{\text{Reading of standard}}$

## **TURBIDIMETRIC MEASUREMENTS**

The basis for calibration or calculation is based on a solution of standard turbidity, and the readings and results are obtained just as with clear solutions. To eliminate loss of determination due to the development of turbidity often the color is developed, the sample can be centrifuged in the macro test tubes using the ordinary 15ml brass centrifuge shield.

## **ACCURACY**

Duplicate readings on the same solution have never been found to differ by more than  $\frac{1}{3}$  of 1 percent of the full scale. This corresponds to  $\frac{1}{3}$  of one scale division on the 100 to 0 scale of the usual Photoelectric Colorimeter.

## **OPERATION**

### **IMPORTANT NOTES**

- Make sure tubes and/or cells are clean and dry. Wipe outside with a lint free tissue or cloth.
- The Colorimeter tubes must always be used with the lettering facing you. Do not use regular test tubes.
- Lamp may be left on all day, but should be shut off at night.
- DO NOT turn the lamp on without a filter in place. This could damage the photo cells.
- The cover over the cell compartment should be closed to keep out extraneous light.
- It is advisable to place the instrument on a table or bench free from vibration, and away from open doors, windows or strong overhead light.

### **PROCEDURE**

1. Make sure there is a filter in place between lamp housing and instrument (See Figure 1, page 4).
2. Pointer (C) on scale to be at 0. If not, adjust with small knob (D) on top of instrument.
3. Plug in instrument.
4. Place tube or cell (E) with distilled water in place.
5. Turn large knob (A) on front of instrument to read 0 on scale (B).
6. Switch on Colorimeter lamp (F).
7. Readjust zero with larger knob (G) on top and further back on instrument.
8. Allow lamp to burn for a few minutes and check zero again.
9. The instrument is now ready for use.
10. To read unknown, remove distilled water and insert tube with unknown solution. The pointer (C) will be deflected from zero. Turn scale knob (A) until pointer has been brought back to zero. The reading on the scale (B) is now the reading of the unknown solution.
11. The concentration of the unknown is then obtained by multiplying scale reading by a factor of a known solution or by reading off a standard curve.

## **MAINTENANCE**

### **CHANGING COLORIMETER LAMP**

1. Disconnect instrument from line.
2. Turn instrument so that back faces you and remove back panel by sliding up.
3. Take note of which way the filament faces; the two loops near the bottom of the filament should face the lens.
4. Push lamp down and turn to left to remove.
5. Place new lamp in socket so the filaments will be properly oriented, push down and turn to the right.

### **CARE OF SLIDE WIRE CONTACT**

To keep dial contact clean, turn dial back and forth a number of times over the scale range by means of the large knob, taking care not to hit the end stops.

## KLETT CLINICAL/INDUSTRIAL COLORIMETER

### PARTS LIST

T37013-0000	Industrial Colorimeter, Glass Cell Mdl. 115V	900-3
T37013-0001	Industrial Colorimeter, Glass Cell Mdl. 220V	
T37013-0010	Solution Cell, 20mm, 40mm	901
T37013-0015	Solution Cell, 20mm, 40mm, Fused	
T37013-0020	Solution Cell, 10mm	902
T37013-0030	Reduction Plate for use w/10mm Cell	903
T37013-0041	Adapter for Round Test Tubes	904 & 904M
93701-2009	Lamp Bulb 110-120V	802-L
37012-1000	Conversion kit 220 to 115V needed on older models for replacement bulbs	
93701-2103	Small Lamp for Dial 110-120V	
93701-2100	Small Lamp for Dial 220V	10S10
T37014-0040	KS-40 Filter	
T37014-0042	KS-42 Filter, Blue	803-B
T37014-0044	KS-44 Filter	
T37014-0047	KS-47 Filter	
T37014-0050	KS-50 Filter	
T37014-0052	KS-52 Filter	
T37014-0054	KS-54 Filter, Green	803-G
T37014-0055	KS-55 Filter	
T37014-0056	KS-56 Filter	
T37014-0059	KS-59 Filter	
T37014-0060	KS-60 Filter	
T37014-0062	KS-62 Filter	
T37014-0064	KS-64 Filter	
T37014-0066	KS-66 Filter, Red	803-R
T37014-0069	KS-69 Filter	
T37014-0070	KS-6225 Filter	
T37013-1015		Klett Industrial Test Kit
93701-2011	Extra frame for 2" Filter	808
93701-2014	Galvanometer	800-9
93701-2010	Photocells (pair)	801-15

## **BIBLIOGRAPHY OF METHODS INORGANIC/ORGANIC**

The list of colorimetric procedures includes the common methods of determination of interest in the field of industrial analysis. In most cases, no modification of the original procedure is required.

Selection of light filters is based on the rule that the proper light filter is one whose spectral transmission is opposite to that of the solution being measured. These are purely arbitrary selections, except in those cases where the authors have specified a definite filter.

Clinical Methods are omitted since the procedures are outlined in detail in the Clinical Manual for the test tube model of the photoelectric colorimeter.

For a comprehensive review of colorimetric methods, the following texts are recommended:

E.B. Sandell  
**Colorimetric Determination of Traces of Metal**  
Interscience Publishers, Inc., New York (1959)

Snell, F.D., and Snell, C.T.  
**Colorimetric Methods of Analysis**  
D. Van Nostrand Company, New York  
Volume 1, Volume 2, Volume 3, Volume 4

Snell, F.D., and Snell, C.T., and Snell, C.A.  
**Colorimetric Methods of Analysis**  
Including Photometric Methods  
D. Van Nostrand Company, New York  
Volume 11A (March 1959)

Yoe, John H.  
**Photometric Chemical analysis/John Wiley & Sons, New York**  
Volume 1 and Volume 2, (1928)

For comprehensive review of colorimetric water methods, the following text is recommended:

**Standard Methods for the Examination of Water and Sewage**  
Tenth Edition  
Published by: American Public Health Association, New York, NY

For a comprehensive review of colorimetric oil methods, the following text is recommended:

**A.S.T.M. Standards**  
Published by: American Society for Testing Materials, Philadelphia, PA

For a comprehensive review of colorimetric food methods, the following text is recommended:

**The Analysis of Foods**  
Andrew L. Winton and Kate Barber Winton  
John Wiley & Sons, New York

**Official Methods of Analysis of the Association of Official Agricultural Chemists, (1958 Edition)**

Published by: Association of Official Agricultural Chemists, Washington

For a comprehensive review of colorimetric vitamin methods, the following texts are recommended:

**Methods of Vitamin Assay**

Published for: The Association of Vitamin Chemists, Inc., by:  
Interscience Publishers, Inc., New York

**Vitamin Methods**

Volume 1, Ed.-Paul Gyorgy  
Published by: Academic Press, Inc., Publishers, New York (1950)

For a comprehensive review of colorimetric soil and plant methods, the following text is recommended:

**Soil and Plant Analysis**

C.S. Piper  
Published by: Interscience Publishers, Inc., New York (1947)

For a comprehensive review of colorimetric steel methods, the following text is recommended:

**Ferrous Analysis, Modern Practice and Theory**

Second Edition  
D.C. Garrett  
Published by: John Wiley & Sons, New York (1953)

For a comprehensive review of colorimetric drug methods, the following text is recommended:

**The Quantitative Analysis of Drugs**

Second Edition  
E.C. Pigott  
Published by Philosophical Library (1953)

References to procedures are listed below in connection with the following materials and products.

**Cement**

Chemical Abstracts-2280-April 20, 1946  
Chemical Abstracts-6596-July 25, 1950

**Cosmetics**

Chemical Abstracts-1705-March 10, 1948

**Beer**

Chemical Abstracts-2533-April 20, 1947  
Chemical Abstracts-1646-February 25, 1950  
Chemical Abstracts-8050-September 10, 1950  
Chemical Abstracts-8594-September 25, 1950

**DDT**

Chemical Abstracts-700-January 25, 1951

**OIL**

Chemical Abstracts-10351-November 10, 1950

Chemical Abstracts-7069-September 10, 1948

**FLUORINE**

Chemical Abstracts-8707-November 20, 1948

Analytical Chemistry-603-April 1951

**FRUIT**

Journal of American Chemical Society-3586-November 1948

Science-May 20, 1949

**GASOLINE**

Analytical Chemistry-April-1948

Analytical Chemistry-805-September 1948

Chemical Abstracts-3153-April 10, 1951

**RUBBER**

Analytical Chemistry-January 1948

Analytical Chemistry-313-February 1951

**COLOR DETERMINATION OF MAGNESIUM IN PLANT TISSUES**

Analytical Chemistry-751-May 1951

**COLOR DETERMINATION OF NITRATES IN PLANT MATERIAL**

Chemical Abstracts-p. 2368-March 25, 1951

**COLOR DETERMINATION OF NICOTINE**

Analytical Chemistry-p. 924-June 1951

**COLOR DETERMINATION OF NUCLEOTIDE IN ACTIN PREPARATION**

Journal of Biological Chemistry-p. 599- August 1951

**COLOR DETERMINATION OF SODIUM AND POTASSIUM**

Chemical Abstracts-p. 1908-March 10, 1951

**COLOR DETERMINATION OF DINITROPHENYL DERMATINE IN TOBACCO**

Journal of Biological Chemistry-p. 727- October 1951

**COLOR DETERMINATION OF COLOR IN TOMATO PASTE**

Chemical Abstracts-p. 2138-March 10, 1950

**TURBIDITY MEASURE OF TOBACCO MOSAIC VIRUS**

Journal of Biological Chemistry-55-May 1951

**SALICYLATE ON CLOTH**

Analytical Chemistry-p. 1170-August 1951

# INORGANIC TESTS

## ALUMINUM

Reagent–Aluminon (aurin tricarboxylic acid)

Dye forms a bright red lake with aluminum. Add gum arabic in determining quantities greater than 1 mg.

Filter–54 or 42

Reference–L.P. Hammett and C.T. Sottery, *J. Am. Chem. Soc.*, **47**, 142 (1925). Yoe and Hill

–*J. Am. Chem. Soc.*, **49** 2395 (1927)

## AMMONIA

Reagent–Nessler's reagent

Develop yellow-brown color

Filter–42

Reference–Snell and Snell, *Colorimetric Methods of Analysis*, D. Van Nostrand Co., Inc., New York, Volume 2, p. 814; H.F. Beeghly, *Ind. Eng. Chem. Anal. Ed.* **14**, 137-40 (1942); Margherita Marzada, *Ann. chim. applicata* **35**, 231-5 (1945)

## ANTIMONY

Reagent–Rhodamine B

Develop red color

Filter–54

Reference–William G. Frederick, *Ind. & Eng. Chem., Anal. Ed.*, **13**, 992 (1941)

## ARSENIC

Reagent–Quinine arseno molybdate

Nephelometric method. Arsenic content of unknown should be between 0.008 and 0.035 mg.

Filter–42

Reference–D. Chouchak–*Analyst*, **47**, 317 (1922)

## ARSENIC

Reagent–Bougault's reagent (acid sodium thiosulfate)

Reduction of metallic arsenic to form a brown color

Filter–54

Reference–A. Amati–*Biochim. terap. sper.* **20**, 523-30 (1933)

## BARIUM

Reagent–Ammonium chromate

Barium precipitated as the chromate, precipitate dissolved and read in the colorimeter. Best range 0.3-1.0 mg.

Develop yellow color.

Filter–54 or 42

Reference–H.A. Frediani and B. J. Babler, *Ind. & Eng. Chem., Anal. Ed.*, **11**, 487-9 (1939)

## BERYLLIUM

Reagent–Quinalizarin

Develop blue color

Filter–42 or 54

Reference–E.B. Sandell, p. 153, *Colorimetric Determination of Traces of Metals*, Interscience Publishers, Inc. New York



## **BISMUTH**

Reagent–Potassium iodide

Solution of bismuth sulfate or bismuth nitrate assumes yellow to orange color. Detects 0.1 mg.

Filter–54 or 42

Reference–C.C. Leonard, J. Pharmacol.

## **BISMUTH**

Reagent–Ammonia cyanide mixture

Bismuth extracted with dithizone solution forms a bright orange color. Potassium thiocyanate prevents interference by other metals. Range 0.005-0.05 mg.

Filter–54 or 42

Reference–D.M. Hubbard-Ind. & Eng. Chem., Anal. Ed., 11, 343-5 (1939)

## **BORON**

Reagent–Quinalizarin

For the determination of boron in soils and plants. Color change from pink to blue as concentration increases. Detects 0.001 mg.

Filter–54

Reference–K.C. Berger and E. Trough-Ind. & Eng. Chem., Anal. Ed., 11, 540-5 (1939)

## **BORON**

Reagent–Curcumin

Solution of boric acid and oxalic acid evaporated to dryness with curcumin. Detects 0.001 to 0.01 mg. Develop red color

Filter–54 or 42

Reference–J.A. Naftel-Ind & Eng. Chem., Anal. Ed., 11, 407-9 (1939)

## **BROMINE**

Reagent–Chlorine water, carbon tetrachloride

Develop yellow to reddish-brown color

Filter–54 or 42

References–Snell and Snell, Colorimetric Methods of Analysis, D. Van Nostrand Co., Inc., New York, Volume 2, p. 723

## **CALCIUM**

Reagent–Alizarin

Calcium alizarinate dissolved in alcohol. Develop purple color.

Filter–54

Reference–P.P. Laidlow and W.W. Payne--Biochem. J., 16, 494-8 (1922)

## **CERIUM**

Reagent–Brucine

Develop red color fading to orange-brown

Filter–47

Reference–E.B. Sandell, p. 386 Colorimetric Determination of Traces of Metals, Interscience Publishers, Inc. New York. Third Edition (1959)

## CHLORINE

Reagent-para-Aminodimethylaniline

Develop purple color, which is stable for 5 minutes. Beer's law does not hold for concentrations greater than 0.65 parts per million.

Filter-54

Reference-D.H. Byers and M.G. Mellon-Ind & Eng. Chem., Anal. Ed., **11**, 202-3 (1939)

## CHROMIUM

Reagent-Diphenyl Carbazide

Develop purple color, sensitive to 1 part per million

Filter-54

Reference-A. Cazeneuve-Analyst, **25**, 231 (1900); G.P. Rowland-Ind. & Eng. Chem., Anal. Ed., **11**, 442-5 (1939)

## COBALT

Reagent-Arsenophosphotungstic acid

Develop blue color-can be determined in the presence of large quantities of nickel

Filter-66

Reference-A. Leiberson-J. Am. Chem. Soc., **52**, 464-5 (1930)

## COPPER

Reagent-Potassium ethyl xanthate

Develop yellow color

Filter-54 or 42

Reference-G.C. Supple and B. Bellis-J. Dairy Sci., **5**, 455 (1922)

## COPPER

Reagent-Sodium diethyldithiocarbamate

Develop golden-brown color. Range 0.025-0.15 mg

Filter-54 or 42

Reference-W.D. McFarlane-Biochem, J., **26**, 1022-33 (1933); D.E. Frear-Ind. & Eng. Chem., Anal. Ed., **11**, 494-5 (1939)

## COPPER

Reagent-Ammonia

Develop intense blue color of cupric ammonium complex-used for the determination of copper in iron and steel

Filter-66

Reference-F.T. Sisco, The Technical Analysis of Steel, McGraw-Hill Co., New York, p.272

## CYANIDE

Reagent-Ferric Chloride

Develop red color

Filter-54

References-Photometric Chemical Analysis, John H. Yoe, John Wiley & Sons, Inc., New York, Volume 1, p. 154, (1928)

## FLUORINE

Reagent-Ferron (7-iodo-8-hydroxyquinoline-5 sulfonic acid), plus ferric chloride, plus HCl)

Green color of ferron-iron reagent assumes yellowish hue when fluorine is present

Filter-42

Reference-J.J. Fahey, Ind. & Eng. Chem. Anal. Ed., **11**, 362-3 (1939)

## GERMANIUM

Reagent–Molydigermanic acid

Develop yellow color

Filter–42

Reference–R.E. Kitson and M.G. Mellon-Ind. & Eng. Chem., Anal. Ed., **16**, 128 (1944)

## IRON

Reagent–Potassium thiocyanate

Develops red color. Oxidize iron to ferric state. Reaction between ferric and thiocyanate ions.

Filter–54

Reference–Snell and Snell, Colorimetric Methods of Analysis, D. Van Nostrand Co., Inc., New York, Volume 1, p. 283

## IRON

Reagent–thioglycoloc (mercaptoacetic) acid

Develops blue-purple color with ferric iron. Develops pink-red color with ferrous iron

Filter–54 for Ferric; 42 for Ferrous

References–Fe-G. Leavell and N.R. Ellis-Ind. & Eng. Chem., Anal. Ed., **6**, 46-7 (1934); Fe-E. Lyons-J. Am. Chem. Soc., **49**, 1916 (1927)

## LEAD

Reagent–Dithizone (diphenylthiocarbazon)

Green color of the reagent turns red

Filter–54

Reference–J.R. Ross and C.C. Lucas-Can. Med. Assoc. J., 29, 649-50 (1933); C.L. Guettel-Ind & Eng. Chem., Anal. Ed., **11**, 639-40 (1939)

## LEAD

Reagent–Stannous chloride & thiocyanate

Lead peroxide converted to molybdate, which gives you an orange color with stannous chloride and thiocyanate

Filter–54 or 42

Reference–S. Feinberg-J. Anal. Chem. **96**, 415-8 (1934)

## MAGNESIUM

Reagent–8-Hydroxyquinoline

Develop green-black pigment

Filter–66

Reference–C.P. Sideris-Ind. & Eng. Chem., Anal. Ed., **12**, 232-3 (1940)

## MANGANESE

Reagent–Potassium metaperiodate

Manganese oxidized to permanganate and purple colored solution measured

Filter–54

Reference–G.P. Roland-Ind. & Eng. Chem., Anal. Ed., **11**, 442-5 (1939) J.P. Mehlig-Ind. & Eng. Chem., Anal. Ed., **11**, 274-7 (1939)

## MERCURY

Reagent–Phosphomolybdotungstic acid

Develop blue color-sensitive to 2 parts per million

Filter–66

Reference–V. Ciocalten and C. Titel--comp. rend. soc. biol., **112**, 621-2 (1933)

## **MOLYBDENUM**

Reagent–Potassium thiocyanate

Develop reddish-brown color. Soluble in butyl acetate, which may be used for extraction. Suitable for determination in steel

Filter–54 or 42

Reference–O.A. Maag and C.H. McCollam-Ind. & Eng. Chem., 17, 524 (1925)

## **NICKEL**

Reagent–Dimethylglyoxime

Develop red color

Filter–54 or 42

Reference–A.P. Rollet-Comp. rend., **183**, 212 (1926); E.B. Sandwell and R.W. Perlich-Ind. & Eng. Chem., Anal. Ed., **11**, 309-11 (1939)

## **NICKEL**

Reagent–Dimethylglyoxime, ammonia

Precipitate nickel with dimethylglyoxime, followed by conversion of precipitate to nickel-ammonia complex.

Develop blue color

Filter–66

References–G.H. Ayres and F. Smith-Ind. & Eng. Chem., Anal. Ed., **11**, 365-6 (1939)

## **OSMIUM**

Reagent–Thiourea

Develop red color

Filter–54

Reference–E.B. Sandell-Ind. & Eng. Chem., Anal. Ed., **16**, 342 (1944)

## **PALLADIUM**

Reagent–p-Nitrosodimethylaniline

Develop red color

Filter–54

Reference–E.B. Sandell p. 716, Colorimetric Determination of Traces of Metals, Interscience Publishers, Inc., New York (1959) Third Edition. Volume III

## **PERCHLORATES**

Reagent–Nitrosodimethylaniline

Develop violet color

Filter–54

Reference–Snell and Snell, Colorimetric Methods of Analysis, D. Van Nostrand Co., Inc., New York, Volume 2, p. 721, Third Edition

## **PHOSPHORUS**

Reagent–Ammonium molybdate

Develop blue color of phosphomolybdate, further methods by reduction of the phosphomolybdate

Filter–66

Reference–Snell and Snell, Colorimetric Methods of Analysis, D. Van Nostrand Co., Inc., New York, Volume 2, p. 478-481, Third Edition

## PLATINUM

Reagent–Stannous chloride

Develop yellow color

Filter–43

Reference–E.B. Sandell, p. 726-728, *Colorimetric Determination of Traces of Metals*, Interscience Publishers, Inc., New York, (1959), Third Edition, Volume III.

## POTASSIUM

Reagent–Dipicrylamine (hexanitrodiphenylamine)

Solution yellow to orange-red. Range 0.01-0.1 mg. Beer's law does not hold for aqueous solutions of potassium dipicrylamine.

Filter–54 or 42

Reference–I.M. Kolthoff and G.H. Bendix-*Ind. & Eng. Chem., Anal. Ed.*, **11**, 94-8 (1939)

## RHENIUM

Reagent–Potassium Thiocyanate

Develop yellow color

Filter–42

Reference–E.B. Sandell, p. 378, *Colorimetric Determination of Traces of Metals*. Interscience Publishers, Inc., New York

## RHODIUM

Reagent–Stannous chloride

Develop yellow color

Filter–42

Reference–E.B. Sandell, p. 386, *Colorimetric Determination of Traces of Metals*, Interscience Publishers, Inc., New York

## SELENIUM

Reagent–Codeine sulfate

Develop blue color-range 0.05–0.5 mg.

Filter–66

Reference–R.A. Gortner and H.B. Lewis-*Ind. & Eng. Chem., Anal. Ed.*, **11**, 198-200 (1939)

## SILICA

Reagent–Ammonium molybdate

Develop yellow color

Filter–54 or 42

Reference–F. Dienert and F. Wandenbulcke, *Compt. rend.*, **176**, 1478, (1923); H.W. Knudson, C. Juday, and V.W. Meloche-*Ind. & Eng. Chem., Anal. Ed.*, **12**, 270-3 (1940)

## SILVER

Reagent–Rhodamine

Develop red-violet color

Filter–54

Reference–E.B. Sandell, p. 395, *Colorimetric Determination of Traces of Metals*, Interscience Publishers, Inc. New York

## SODIUM

Reagent–Uranyl acetate–zinc acetate

Precipitate uranyl zinc sodium acetate–measure color developed by sulfosalicylic acid and sodium acetate

Filter–42

Reference–M.C. Darnell, Jr., and B.S. Walker–Ind. & Eng. Chem., Anal. Ed., **12**, 242-44 (1940)

## SULFIDE

Reagent–p.Aminodimethylaniline

Sulfur present as sulfedi decomposed with acid yields methylene blue

Filter–66

Reference–Snell and Snell, Colorimetric Methods of Analysis, D. Van Nostrand Co., Inc., New York, Volume 1, p. 593

## THALLIUM

Reagent–Phosphomolybdic acid

Develop yellow solution

Filter–54 or 42

Reference–F. Pavelka and H. Morth–Mikrochemie, **5**, 30-3 (1932)

## TIN

Reagent–Ammonium molybdate

Develop blue color–detects 0.1 mg. tin

Filter–66

Reference–G.F. Huttig–Chem. Ztg. **47**, 341 (1923)

## TITANIUM

Reagent–Perchloric acid

Develop yellow color

Filter–54 or 42

Reference–Louis Silverman–Ind. & Eng. Chem., Anal. Ed., **14**, 791 (1942)

## TUNGSTEN

Reagent–Hydroquinone

Develop red color when dissolved in sulfuric acid

Filter–54 or 42

Reference–G. Heene–Z. Angew. Chem.

## URANIUM

Reagent–Hydrogen peroxide

Develop yellow color

Filter–42 preferred (also 47 or 44)

Reference–E.B. Sandell, p. 915-916, Colorimetric Determination of Traces of Metals, Interscience Publishers, Inc., New York. Third Edition, Volume III, (1959)

## VANADIUM

Reagent–Phosphotungstic acid

Develop yellow color

Filter–42 or 54

Reference–E.R. Wright and M.G. Mellor–Ind. & Eng. Chem., Anal. Ed., **9**, 251 (1937)

## ZINC

Reagent-Dithizone

Develop red color in carbon tetrachloride. Determine 0.001–1.0 mg of zinc.

Filter-54 or 42

Reference-W. Dackert-Z. Anal. Chem., **100**, 386-90 (1935)

## ZIRCONIUM

Reagent-p-Demethylaminoazophenylarsonic acid

Develop yellow color

Filter-42

Reference-W.G. Hayes and E.W. Jones-Ind. & Eng. Chem., Anal. Ed., **0**, 603 (1941)

# ORGANIC TESTS

## ACETYLENE

Reagent—Ammoniacal cuprous chloride

Develop red color

Filter—54 or 42

Reference—Photometric Chemical Analysis, John H. Yoe, Volume I p.402, John Wiley & Sons, Inc., New York, (1928)

## BENZALDEHYDE

Reagent—Schiff's reagent

Develop violet color

Filter—54

Reference—H.N. Alyea and H.L. Backstrom-J. Am. Chem. Soc., **51**, 90-109 (1929)

## BENZOIC ACID

Reagent—Copper sulfate

Benzoic acid oxidized to salicylic acid. Develop red color

Filter—42

Reference—J.R. Nicholls-Analyst, **53**, 19-29 (1928)

## BETA-CAROTENE

Reagent—extract with petroleum ether

Read intrinsic color in colorimeter

Filter—authors use filter transmitting at 450 nanometers. Combination of Cenco lantern blue #554, and Corning Noviol A #0.038

Reference—R.O. Brooke, S.W. Tyler, and W.S. Baker-Ind. Eng. Chem., Anal. Ed., **11**, 104-6 (1939); D.M. Hegsted, J.S. Porter, and W.H. Peterson-Ind. & Eng. Chem., Anal. Ed., **11**, 256-8 (1939); V.E. Munsey-J. Assoc. Official Agr. Chem., **20**, 459 (1937); H.G. Petering, W. Wolman, and R.P. Hibbard, Ind. & Eng. Chem., Anal. Ed., **12**, 148-51 (1940); C.L. Shrewsbury, H.R. Kraybill, and R.R. Winthrow-Ind. & Eng. Chem., Anal. Ed., **10**, 253 (1938)

## CHLOROPHYLL

Reagent—extract with acetone or alcohol

Read intrinsic color in colorimeter

Filter—66

Reference—H.G. Petering, W. Wolman, and R.P. Hibbard-Ind. & Eng. Chem., Anal. Ed., **12**, 148-51 (1940)

## ETHYL ALCOHOL

Reagent—Potassium bichromate

Oxidize ethyl alcohol to acetic acid—color varies from blue-green to green-yellow. Does not follow Beer's law

Filter—66

Reference—R. Fontayne and P. desmet, Mikrochemie, **13**, 289-403 (1933)

## FORMALDEHYDE

Reagent—Phenylhydrazine hydrochloride

Forms a violet colored compound

Filter—54

Reference—S.B. Schryver and C.C. Wood, Analyst, **45**, 164-70 (1920)



## FORMIC ACID

Reagent–Rosaniline or fuchsin reagent

Develop yellow color

Filter–42

Reference–L.A. Wetlaufer, F.L. Van Natta and H.B. Quattlebaum–Ind. & Eng. Chem., Anal. Ed., **11**, 438-0 (1939)

## FURFURAL

Reagent–Ferric chloride

Develop red to blue-violet color

Filter–54

Reference–Snell and Snell, Colorimetric Methods of Analysis, D. Van Nostrand Co., Inc. New York, Volume 3, p. 303-305

## HYDROGEN PEROXIDE

Reagent–Ammonia molybdate

Develop yellow color

Filter–42

Reference–M.L. Isaacs, J. Am. Chem. Soc., **44**, 1662 (1922)

## INDOLE

Reagent–Dimethylaminobenzaldehyde

Extract indole (0.001-0.1mg) with chloroform. Develop purple-red color

Filter–54

Reference–L.H. Chernoff–Ind. & Eng. Chem., Anal. Ed., **12**, 273-4 (1940)

## MONOMITROPARAFFINS (PRIMARY)

Reagent–Ferric chloride

Determines monomitroparaffins except methane

Filter–Wratten 65 A in B glass

Reference–E.W. Scott and J.F. Treon–Ind. & Eng. Chem., Anal. Ed., **12**, 189 (1940)

## PHENOLS

Reagent–Acetic acid, nitric acid, and sulfuric acid

Develop yellow color

Filter–42

Reference–L.A. Wetlaufer, F.L. Van Natta and H.B. Quattlebaum–Ind. & Eng. Chem., Anal. Ed., **11**, 438-0 (1939)

## SALICYLIC ACID

Reagent–Ferric chloride

Develop violet color

Filter–54

Reference–H. Riffert and H. Keller–Z. Untersuch, Lebensm, **68**, 113-38 (1934)

## UREA

Reagent–Unrease

Then determine ammonia (Folin's method)

Filter–542

Reference–P.A. Racocot and H.C. Lythgoe–Ind. & Eng. Chem., Anal. Ed., **11**, 512-15 (1939)

## % TRANSMITTANCE INFORMATION

The Klett-Summerson Colorimeter scale is graduated in units proportional to the optical density. The actual numerical values represent the optical density divided by two, with the decimal point omitted. For example, a scale reading of 250 corresponds to an optical density of 0.500. The formula relating scale reading and optical density is as follows:

$$(1000/2) \times D = R$$

where D is the density, and R is the reading.

It is sometimes desired to obtain scale reading in terms of **percent transmittance** (less correctly called **percent transmission**). For this purpose, the accompanying table may be used. From this table the optical density and the percent transmittance corresponding to any Klett-Summerson scale reading may be readily obtained.

It is also possible to calculate the percent transmittance value corresponding to any Klett-Summerson scale reading. This is done as follows:

1. Obtain the optical density, by multiplying the scale reading by 0.002.
2. Subtract the optical density value from 2.000. The result is the logarithm of the percent transmittance. From logarithm tables, obtain the value of the percent transmittance.

Example    Scale reading = 150  
              Optical density =  $150 \times 0.002 = 0.300$   
              log % transmittance =  $2.000 - 0.300 = 1.7$   
              % transmittance = 50.2%

The accompanying table affords a convenient way of obtaining optical density values from scale readings.

## % TRANSMITTANCE CHART

READING	OPTICAL DENSITY	% TRANSMITTANCE	READING	OPTICAL DENSITY	% TRANSMITTANCE	READING	OPTICAL DENSITY	% TRANSMITTANCE
0.0	0.000	100	90.5	0.181	66	247.5	0.495	32
2.0	0.004	99	93.5	0.187	65	254.5	0.509	31
4.5	0.009	98	97.0	0.194	64	261.5	0.523	30
6.5	0.013	97	100.5	0.201	63	269.0	0.538	29
9.0	0.018	96	104.0	0.208	62	276.0	0.552	28
11.0	0.022	95	107.5	0.215	61	284.5	0.569	27
13.5	0.027	94	111.0	0.222	60	292.5	0.585	26
16.0	0.032	93	114.5	0.229	59	301.0	0.602	25
18.0	0.036	92	118.5	0.237	58	310.0	0.620	24
20.5	0.041	91	122.0	0.244	57	319.0	0.638	23
23.0	0.046	90	126.0	0.252	56	329.0	0.658	22
25.5	0.051	89	130.0	0.260	55	339.0	0.678	21
28.0	0.056	88	134.0	0.268	54	349.5	0.699	20
30.5	0.061	87	138.0	0.276	53	360.5	0.721	19
33.0	0.066	86	142.0	0.284	52	372.5	0.745	18
35.5	0.071	85	146.0	0.292	51	385.0	0.770	17
38.0	0.076	84	150.5	0.301	50	398.0	0.796	16
40.5	0.081	83	155.0	0.310	49	412.0	0.824	15
43.0	0.086	82	159.5	0.319	48	425.0	0.850	14
46.0	0.092	81	164.0	0.328	47	443.0	0.886	13
48.5	0.097	80	168.5	0.337	46	460.5	0.921	12
51.0	0.102	79	173.5	0.347	45	479.5	0.959	11
54.0	0.108	78	178.5	0.357	44	500.0	1.000	10
57.0	0.114	77	183.5	0.367	43	523.0	1.046	9
59.5	0.119	76	188.5	0.377	42	548.5	1.097	8
62.5	0.125	75	193.5	0.387	41	577.5	1.155	7
65.5	0.131	74	199.0	0.398	40	611.0	1.222	6
68.5	0.137	73	204.5	0.409	39	650.5	1.301	5
71.5	0.143	72	210.0	0.420	38	699.0	1.398	5
74.5	0.149	71	216.0	0.432	37	761.5	1.523	3
77.5	0.155	70	222.0	0.444	36	849.5	1.699	2
80.5	0.161	69	228.0	0.456	35	1000.0	2.000	1
84.0	0.168	68	234.5	0.469	34			
87.0	0.174	67	241.0	0.482	33			







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# I N S T R U C T I O N S



## 220VAC to 115VAC Conversion Kit for Klett

Catalog No. 37012-1000

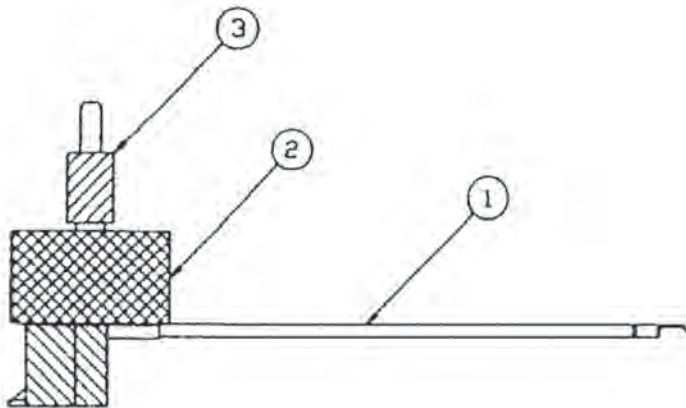
This conversion kit is for using 115 volt Klett Colorimeter with 220 volt supply.

### WARNING

- Turn off electrical power before beginning the installation.
- Never use the Conversion Kit without proper grounding.
- Klett must be used with grounded outlet.

This Conversion Kit includes these three major components:

1. Grounding Adapter
2. 220VAC to 115VAC Power Converter
3. One Projection lamp and one Dial lamp



### INSTALLATION

Installation must be done according to the applicable electrical safety code of the local authority having jurisdiction over the installation.

If the Klett is a 220 volt unit, replace all 220 volt bulbs with the 115 volt bulbs. This makes the Klett a 220 volt unit. After replacing the bulbs, do the following steps:

1. Turn off electrical power.
2. Plug in the Grounding Adapter into the Power Converter.
3. Attach the Grounding Adapter green wire to outlet ground.
4. Plug in the Power Converter into the 220 volt outlet.
5. Turn on the electrical power. Power output at the Grounded Adapter should be 115VAC.
6. Plug in the 115VAC Klett Colorimeter to the Grounded Adapter.
7. To operate the Klett Colorimeter, follow the Klett Colorimeter Operating Instructions

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# I N S T R U C T I O N S



## Preparing KS Machine for Operation

### To Prepare Klett-Summerson Machine for Operation

1. **Select proper place** for instrument: table or bench away from a window or strong direct overhead light, and is free from vibration.
2. **Connect to appropriate outlet** (110-115 V AC/DC or 220-230 V AC/DC), be sure circuit is not overloaded or subject to power surges.
3. **Check Galvanometer Zero:** check mechanical zero positions of galvanometer. To zero pointer, turn knob on galvanometer until pointer rests on zero.
4. **Insert Filter:** Put proper filter all the way into filter holder to avoid stray light which may cause faulty readings (follow the directions in the instruction booklet). Slide filter holder into the compartment directly in front of the lamp housing.
5. **Set Potentiometer** at zero.
6. **Insert Blank Sample:** Place test tubes or solution cell containing distilled water or blank solution into the holder.
7. **Turn on:** light switch and galvanometer switch.
8. **Zero Galvanometer:** galvanometer needle will deflect; turn the knob on the shutter to bring it back to zero. The instrument is now ready to receive samples. (See Instruction Booklet for further steps).

### To Change the lamp:

1. Unplug the instrument.
2. Turn instrument so that back faces you and remove louvered back panel by sliding up.
3. Take note of which way the filament faces; the two loops near the bottom of the filament should face the lens.
4. Push lamp down and turn left to remove.
5. Place new lamp in socket so the filaments are properly oriented, push down and turn to the right.

**To Keep Slide Wire Contact Clean:** use the large knob to turn the dial back and forth over the full scale range. Do this a number times, taking care not to hit the end stops.

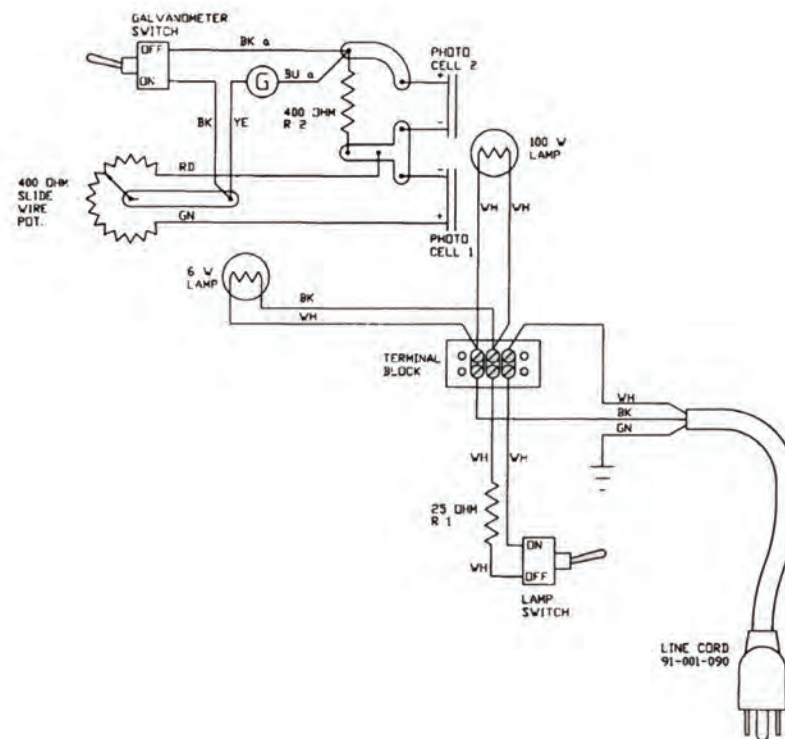
**To Check Galvanometer Switch:** If readings are faulty, turn the galvanometer on and off a number of times.

**Test tubes** are selected for outside dimensions and are interchangeable. For further information, read the instructions booklet carefully.

### Galvanometer

The galvanometer is a suspension wire type instrument and is sensitive to shock and vibration. Also excessive air currents may cause the pointer to move.

Set instrument on 3/4" sponge rubber pad of absorbing tissue. Instrument should be placed in selected spot, away from vibration, open doors and windows.



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