Minipette System : A Simple and Convenient Ultramicro Technique for Routine Clinical Chemistry (Part I)

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Procedures for routine clinical laboratory tests have made remarkable advance since World War II, but it appears that the progress is presently being directed to two specific areas. One is toward automation of laboratory tests, such as the auto-analyzer and the robot chemist whereby all quantitative procedures are performed automatically by instruments without manual aid[1]. The other is to ultramicronization of quantitative procedures which was developed so as to provide adequate chemical test service to critically ill patients and children from whom it is generally difficult to draw blood[2].

From the summer to fall of 1964, one of the authors made an inspection tour of clinical laboratories of various hospitals in Europe and the United States and noted that the laboratories in Switzerland, England, France, Germany and the United States were being automated at a highly rapid pace. That is, most of the laboratories visited were equipped with at least 4 auto-analyzers, while some had as many as 10 units, with which routine clinical biochemical tests were being performed. However, ultramicro procedures were not being fully utilized, and there were even some laboratories where ultramicro apparatuses were left standing unused.

Judging from this, one may gain the impression that future clinical chemistry tests will all be processed by automation, but actually there is a great bottleneck which hinders this. That is, automation requires an enormous investment. Its merits cannot be demonstrated unless a large number of auto-analyzers and robot chemists are installed. Efficiency of a clinical chemistry laboratory cannot be enhanced greatly by the installment of only one auto-analyzer, but at least 4 units are required. In order to realize this it would require an expenditure in excess of 10 million yen. There are not many hospitals in Japan that can afford such an investment. Further great operational costs will be required for purchase of the large quantities of reagents that will be consumed. In addition, in order that such apparatuses may be put to ideal operation, it will be necessary to have experienced technicians to monitor the units. As the apparatus does not have a mind of its own to function, it can readily be imagined that the operator will experience considerable hardship.

On the other hand, if the techniques of ultramicro procedures were dexterously

developed, it would make determinations most economical because the initial output for equipment would be small and the procedures could be carried out with a limited maintenance expenditure. Further, when ultramicro procedures are used, valuable diagnostic information can be readily obtained, as a large variety of tests can be performed at the same time with a small volume of sample material. That is, as explained earlier, if such procedures are adopted, it should be possible to greatly improve the clinical chemistry service to the patiemts in the field of pediatrics as well as to severely sick from whom it is difficult to draw blood. However, wide acceptance of the procedures are hindered by such facts as greater care being required by the technician than in the case of microtechniques because of the extremely small quantity of the sample manipulated and also it often necessitates the procurement of special laboratory space and apparatuses. In other words, the conventional ultramicro procedure involves the use of minute amounts of sample material to which a small quantity of reagent is added to produce color, and determination is carried out on this small quantity of colored solution by use of a special colorimeter. Adopting such methods which cause heavy mental strain as routine procedures is not wise. This is the reason why despite the fact that the need is keenly felt many laboratories have been hesitant about adopting this system.

The authors have made great efforts over a long period of time to develop ultramicro procedures which can be used as routine tests without requiring efforts any greater than those for conventional micro-analysis procedures. Recently, we have been successful in attaining satisfactory results in the application to routine tests. Our ultramicro method devised for routine tests are introduced in the following sections.

ULTRAMICRO QUANTITATIVE METHODS

Chemical analyses using samples of less than 0.1 ml in volume are referred to as ultramicro quantitative methods. The volume of 1 kg of water at 4°C (temperature of maximum density) is termed 1 liter. One thousandth of 1 l is called 1 ml, and 1/1000 of 1 ml is 1 μl or 1λ*.

Analyses using such minute samples were first introduced during the last century, but earnest efforts toward development began in the 1930's. In 1878, Gowers[3] collected blood in a 0.02 ml = 20 μl = 20λ capillary pipet and made quantitative determination of hemoglobin. However, this was an exceptional case. During 1912 to 1922, Pregl and Emich[4] of Austria developed techniques to analyse organic and inorganic substances weighing less than 1 mg. At that time clinical chemistry procedures still required 1 to 10 ml of sample material per item.

*By ultramicro quantitative methods, analyses of samples less than 100λ are carried out, but in many instances the actual volume of the samples taken is 10 to 20λ.

Therefore, when several types of quantitative analyses had to be carried out on the blood components at the same time a volume of several ten ml of blood was needed.

Ultramicro quantitative techniques in the strict sense of the term, actually started being used after 1945, and it may be said that they were devised because of the necessity mainly for the examination and treatment of pediatric patients. That is, Sobel[5], a pioneer in this field, who reported on the techniques at the American Chemical Society in 1945, and subsequently a series of outstanding books on ultramicro clinical chemistry were written by Kirk[6], Caraway[7], O'Brien[8], Natelson[9] and Knights[10].

Saito[11] who studied under Sobel, introduced the methods and techniques to Japan, and has devoted himself to this day towards their dissemination. Nevertheless, there are still many people who consider that the ultramicro methods are to be employed only in special cases and they are useless in routine works.

PRACTICAL ULTRAMICRO QUANTITAIVE METHODS AND THEIR REQUIREMENT

If the ultramicro quantitative methods are to be used as routine clinical tests, it is at first necessary to develop pipets with which ultramicro samples can be measured with ease and simple apparatus by which small volumes of reagents can be accurately added to the sample.

As colorimetry is a more efficient method than titrimetry, the former method (colorimetry) is preferred to the latter method (titrimetry) as the final procedure in ultramicro analyses. The same, of course, would apply to micro procedures as well. In order to do this, it would be necessary to devise a convenient-to-handle photo-electric colorimeter with which it would be possible to accurately determine the extinction of a colored solution in a comparatively small, but yet easy to handle volume (i. e. 1 ml).

It is also necessary to select a colorimetric procedure by which it would be possible to take readings of the colored solution immediately after adding reagents to the sample. This would allow the processing to be made not only quickly and with ease, but also would keep quantitative errors small.

It would be inconvenient if the size of the test tubes for reaction were as large as those used in micro methods. However, on the other hand, if they are too small, they would be difficult to handle. Furthermore, special considerations must be made for the washing of such comparatively small tubes so that a large number can be available for use at one time.

Mixing after the addition of reagents under the micro method is carried out by such procedures as inversion and stirring, but under the ultramicro method a simple device must be considered whereby the solution in the small test tube can be

mixed thoroughly without spilling.

In quantitative methods where reagents must be added to the sample to induce precipitation for the purpose of obtaining supernatant liquid (e.g. deproteinization), it is not possible to filter the liquid through filter paper because the volume is so small. Therefore, in such cases the supernatant is collected by centrifugation. In order to avoid mental strain due to the conduct of the procedures, it is desirable to utilize the ordinary centrifuge used for micro methods. It would be wise not to procure special equipment.

Thus, as mentioned above, there are many conditions that must be met in the conduct of "practical ultramicro methods", but these have been emphasized by Saito[11], and the authors find themselves in complete accord with his opinion.

BASIC PROCEDURES

1. Collection of blood and separation of serum

a. When blood was collected originally for the purpose of performing micro quantitation, but the procedure was subsequently changed to an ultramicro method:

For routine test, 2–3 ml of blood is drawn from the antecubital vein using a dry syringe. About 0.5 ml is immediately transferred into a viale containing dried Heller-Paul's oxalate mixture (one drop), mixed throughly, and the remainder is introduced into a round-bottomed centrifuge tube No. 2 (12×70 mm) for separation of the serum. The blood put into the vial is used for determination of hemoglobin (Hb) and hematocrit (Ht) values, while the serum in the centrifuge tube is used for quantitation of the serum chemical components.

When the contents of the tube has been coagulated and serum begins to separate, the tube is subjected to a centrifuge where it undergoes regular centrifugation (2500 rpm for 5 minutes). Serum is drawn into a 2 ml narrow tipped pipet (fitted with a rubber cap)* and transferred to a small test tube No. 1 (10×70 mm). The serum may be used as test material for about 2 days if the tube is kept in the freezing chamber of a refrigerator with its top (mouth) covered with a piece of parafilm. When the serum is preserved in such fasion, there is hardly any change in the quantitative value for even such vulnerable components as blood sugar**.

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*Any serum that remains in the pipet can be completely removed by touching the tip to a piece of gauze, squeezing the cap and allowing it to be absorbed by the gauze. All serum remaining in the pipet should be thus removed before working on the next sample of serum.

**However, as a rule, one should form the habit of completing quantitation of separated serum within the same day. This is safer and will best serve the purpose of the test.

b. How to collect separated serum (How to use the serum collecting apparatus[12]):

The procedure for transferring serum separated from blood by centrifugation into separate test tubes appears simple, but it necessitates having available the same number of sterile pipets as the samples, which constitutes quite a problem for laboratories that process a large number of samples. As a means of avoiding such complications, it is convenient and enhances efficiency to use the serum collecting apparatus* described hereunder.

Principle and structure (Figures 1 and 2)

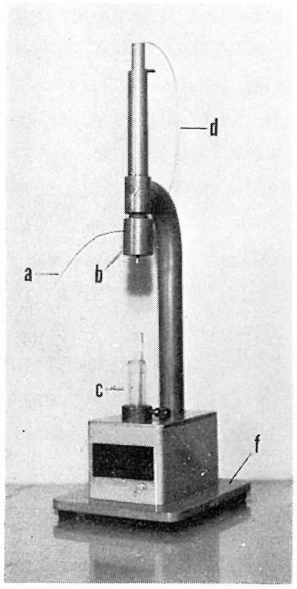

Fig. 1.
Full View of Serum Collector
a–Nozzle, b–Cap, c–Test tube receptacle, d–Intake pipe, e–Drainage pipe (cannot be seen), f–Stand.

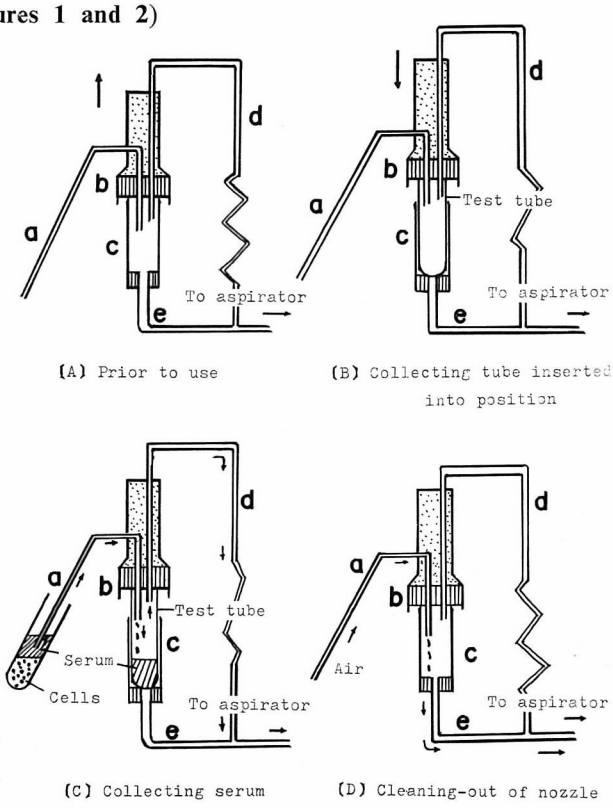

Fig. 2. Construction and Use of Serum Collector
a–Nozzle, b–Cap, c–Test tube receptacle, d–Intake pipe, e–Drainage pipe.

The apparatus has been designed so that negative pressure of the aspirator is led to the nozzle and the serum alone is drawn up without disturbing the cells and is delivered into a separate tube. After this process, air is drawn in through the nozzle to automatically clean-out the bore. The construction of the apparatus is as shown in Figure 1 and 2.–〔A〕

*Commercially available from Fuji Riken, 2-6-4 Yusihma, Bunkyo-ku, Tokyo.

Procedure

The water cock is opened to activate the aspirator, cap (b) is pushed up and turned to the left (Fig. 1). A test tube to which serum is to be transferred (sample collecting test tube) is inserted into test tube receptacle (c)—the apparatus is so designed that the bottom of the test tube will close off the upper end of drainage pipe (e). When cap (b) is turned to the right, it will be forced down so that the rubber seal fitted into the cap will be tightly seated against the mouth of the test tube and the air within the tube will be suctioned out by aspirator pipe (d) creating negative pressure [B].

Next, when the serum portion (usually the supernate) in a test tube containing blood which has been centrifuged is brought into contact with the tip of nozzle (a), the serum will be automatically drawn into collecting test tube [C].

After collection of serum has been completed and the collecting tube is removed, serum remaining in the nozzle can be disposed of by placing the cap (b) tightly against tube receptacle (c), drawing the serum into it and expelling it through drainage pipe (e) [D].

Aspiration is continued in this state for a few seconds after which the cap is again pushed upward and turned to the left. Then both ends of the nozzle are wiped off with a clean piece of gauze.

By bringing centrifuge tubes containing blood samples to the apparatus and repeating the above procedures, it is no longer necessary to have as many pipets as the number of samples and there is no fear of sucking serum into the mouth. However, if the suction power of the aspirator is too large, there is a possibility of drawing up cells. Therefore, it is important to adjust the suction power properly in advance.

The use of this apparatus is not only limited to clinical chemistry tests, but is versatile, and is of particular usefulness in serum separation in the Wassermann reaction test.

c. When blood was collected originally for the purpose of performing ultramicro procedures (Fig. 3, 4):

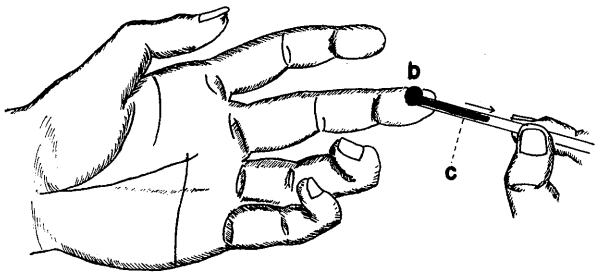

Fig. 3. Collecting blood (b) from the finger tip using a glass capillary tube (C)

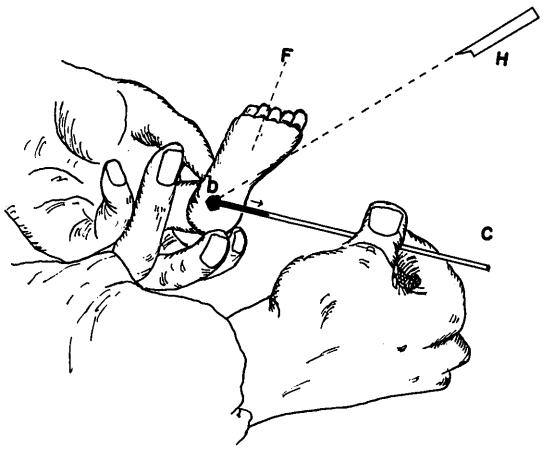

Fig. 4. Collecting blood from the heel (F) of an infant
Prick the heel with a hemolet (H) and collect blood in a glass capillary tube (C) beginning with the second drop.

In the case of children, critically ill patients and obese individuals in whom blood cannot be drawn from antecubital veins, a finger tip is punctured with a hemolet* and blood that flows from the site is collected in a capillary tube. It makes no difference which finger (in the case of infants, the heel) is used. First wipe the site clean with a pledget of cotton soaked in 60–70 % alcohol, wipe it again with a dry sterile piece of gauze and puncture the site with a hemolet. When the first drop of blood appears (if it does not, squeeze the site gently with the fingers), wipe it off with a piece of gauze and collect from the second drop by touching the tip of the capillary tube (bore 2.0 mm, length 11 cm) to the drop allowing the blood to enter the tube through capillarity. Allow blood to run about 3/4 of the way up the tube, and stick the opposite end into a box of crit-seal (used for CRP tests)**. Crit-seal will be forced into the tube and serve as a plug. After the end has been thus sealed, remove the tube from the crit-seal*** (Fig. 5).

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*Commercially available from Futaba Shoji Co., Ltd. (Shutsubou Bldg., 1-1 Kanda Sarugaku-cho, Chiyoda-ku, Tokyo) and Midori Juji (1, Gamomachi 3 chome, Joto-ku, Osaka).

**The tip of the capillary tube by which blood was collected is tilted slightly upward to bring the blood down a little toward the other end. Next, the other end is covered by the index finger and the collector tip is stuck into oil clay (or crit-seal). This allows the tube to be stuck into the crit-seal without any loss of blood. Crit-seal can be obtained commercially from Fuji Kogyo Co. (4-1-14 Yushima, Bunkyo-ku, Tokyo).

***If the tube is removed recklessly from the crit-seal, the plug may come out. Be sure to cover the open end with the index finger, hold the tube between the thumb and middle finger and rotate the tube before removing from crit-seal (Fig. 5). It will be possible to seal the tube perfectly with a little practice.

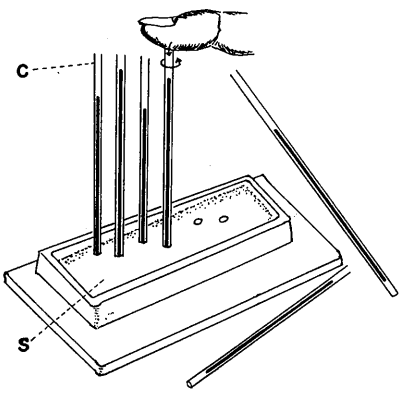

Fig. 5. Collection of blood into capillary tube (C)
The capillary tube in which blood has been collected is stuck upright into a box of Crit-seal (S). The open end of the tube is covered with the index finger, rotated in an anticlockwise direction and removed from the Crit-seal which will be forced into the tube and serve as a plug.

The capillary tube is placed into the usual round-bottomed centrifuge tube together with other tubes*, balanced and spun down (1500 rpm, 10 min.). Serum and clot material will be well separated, and can be immediately sent for volumetric measurement.

d. Collecton of blood directly into a micro polyetylene centrifuge tube (Coleman).

A micro centrifuge tube (Fig. 6) is used to collect blood in lieu of the glass capillary tube described in c).

A blood drawing cap is fitted onto the mouth of the micro centrifuge tube making it air tight. The cap has an aspirator tip (p) and a vinyl tube of adequate length (50 cm). The drop of blood is touched by aspirator tip (p), and when negative pressure is applied on the tube by slight mouth suction, blood will be drawn into the centrifuge tube through pipe (pp').

When blood runs about 4/5 of the way up the centrifuge tube, stop the suction, remove the blood drawing cap and insert plug (ca) into the tube. Spinning by a micro centrifuge for 2 minutes at 1300 rpm will bring about separation of serum.

2. Pipetting the Samples...Sanz Ultramicro Pipet[13]**

Pipetting the sample in ultramicro determinations is different from micro determinations and thus requires special devices. In the past, glass ultramicro

*The blood in a tube sealed with clay or crit-seal alone will leak out if it is put in a centrifuge without further protection, but when a piece of rubber or a rubber cap is placed in the end of a round bottomed centrifuge tube to make it flat, they can be spun down without any added protection. Good separation of serum and clot material can be at attained in such case at 2500 rpm for 5 minutes.

**Commercially available from Fuji Kogyo (1-14, Yushima 4-chome, Bunkyo-ku, Tokyo).

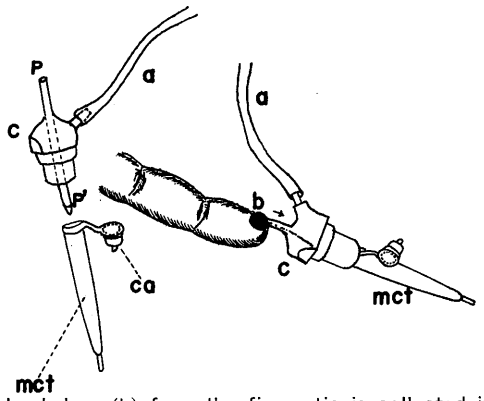

Fig. 6. blood drop (b) from the finger tip is collected in a micro-centrifuge tube (mct).

The micro-centrifuge tube is fitted with cap (c). A polyethylene pipe (pp') penetrates the cap and suction can be applied through the vinyl pipe (a) on the side. After blood has been drawn, the microcentrifuge tube is removed from the cap and plug (ca) is inserted into the tube.

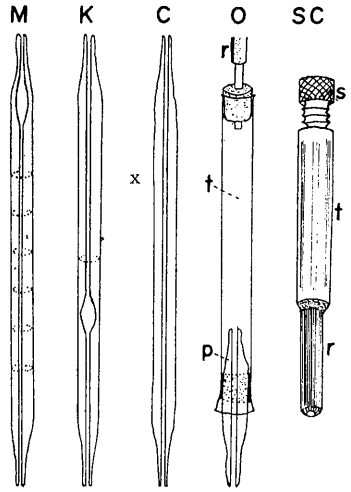

Fig. 7. Pipets (capacity up to 50λ) used for ultramicro determination of serum (blood).

C: Constriction pipet (as the liquid drawn into the pipet readily stops at the constricted section X. When blown out, it is possible to measure the amount of liquid in the bore beyond this section). K: Kirk type. M: Mohr type. O: Overflow pipet (a pipet p of fixed capacity is inserted into tube t and suction by mouth is applied to rubber tube r. Any liquid in excess of the capacity of pipet p will overflow and collect in t. Therefore, it is possible to measure an amount of sample corresponding to the capacity of bore p. When the liquid is blown out into a container, that an amount of sample equivalent to the capacity of the bore has been measured).
SC: Screw cap (screw s is fitted into metal tube t. Vaseline is applied around the screw so that air will not leak out. A firm piece of rubber tube r is fitted to the tip of the tube, and pipets C, K or M are inserted into it. When the screw cap is retreated, sample material will be drawn up into the pipet, and when advanced the sample which has been drawn up is expelled).

pipets (20λ, 50λ, etc.) have been used for this purpose. That is, screw caps were

placed upon the end of the Mohr type pipets which is a straight piece of tubing or on the Kirk type in which there is a round bulb. By slowly loosening (receding) the cap screw the sample is drawn into the pipet up to a predetermined level, and is expelled into a test tube by tightening (pushing forward) the cap screw (Fig. 7). However, such a procedure does not permit speedy measuring of the samples and, as the manipulation requires much attention, it results in great nervous strain giving the worker considerable fatigue when it is necessary to process a large number of samples in succession. It is felt that one of the reasons why ultramicro determination had difficulty in being accepted as a routine test was due to the lack of efficiency in measuring the sample as described above. The procedure was improved considerably with the use of the Lang-Levy constriction pipets (Fig. 7), but it was still impossible to achieve sufficiently satisfactory efficiency.

However, this difficulty was overcome by Sanz's invention (1957)[13]. Sanz's ultramicro pipet (Figure 8) consists of a polyethylene bottle and a small diameter polyethylene tube (pipet) inserted into the cap of the dome. The volume of the bore is of various sizes such as 10, 20, 50 and 100λ. With this device it is possible to draw in a predetermined amount of the sample, and transfer it to another container by blowing out the contents. This pipet is handled in the following manner after first removing polyethylene tube (T) that is in the bottle*.

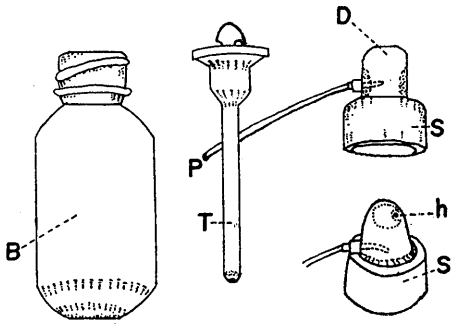

Fig. 8. Sanz Pipet (Description of various parts).
B: Bottle, D: Dome (the wall is transparent and the contents can be observed), S: Cap (fits into the screw grooves of the mouth of the bottle to make it air tight), T: This tube is inserted into B and capped with S. When reagent is put into B, it will move up T and enter into pipet P. T is used only when measuring reagents. h: air hole, p: pipet.

1) The bottle is held between the thumb and middle finger as shown in Fig. 9–1) leaving the index finger free. Pressure is applied to the bottle by lightly

*This polyethylene tube T is shown in Fig. 8. This is used when deliverring predetermined amounts (e.g. 20λ, 50λ, etc.) of reagent in succession into small test tubes. When measuring serum, it is difficult to operate with this inserted in the bottle. Therefore, this tube must be removed, and the Sanz pipet is reassembled with the pipet, dome and bottle only.

squeezing it with the thumb and middle finger.

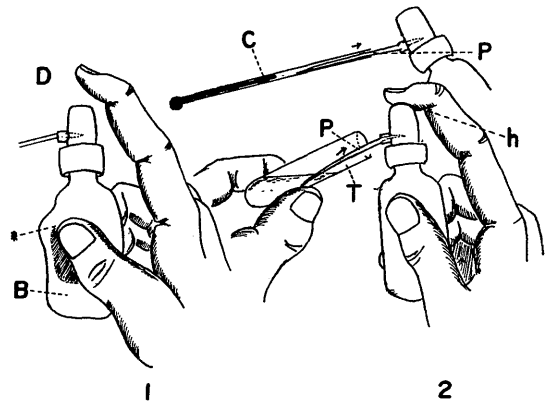

Fig. 9. Taking the Sample with a Sanz Pipet

1) Squeeze the bottle between the thumb and middle finger.

2) When taking the sample from test tube T or glass capillary c, first cover air hole h of the dome and insert the tip of the pipet p into the serum sample in a capillary or a test tube and gradually release the pressure. The sample will be drawn into p and slightly overflow into the dome. The pressure on the air hole applied by the index finger is released immediately and p will be filled with the sample.

2) The small test tube containing the sample is held in the left hand, tilted and the tip of the Sanz pipet is inserted into the sample. The air hole at the top of the dome is covered with the tip of the right index finger, and the pressure* applied to the bottle with the thumb and middle finger is slightly eased. The liquid in the test tube will fill the tube, oveflow and start to run into the bottle (the tip of the pipet to the inside of the dome can be observed well through the wall of the dome). At this point the index finger covering the pore in the dome is released, and the sample will remain stopped in the pipet. The pipet is then wiped with a piece of gauze**.

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*If, on the contrary, pressure is applied by the thumb and middle finger, air will be blown into the sample causing bubbles and mixing of the sample, hindering measurement. Care should be exercised.

**If any of the sample adheres around the outside of the tip of the pipet (p of Fig. 9-2)), it will be transferred to the test tube invalidating the measurement. Therefore, a piece of gauze should be held against the mid-section of the pipet and used to wipe off lightly and quickly after each measuring. This will make it possible to limit the sample to only what was aspirated in the pipet so that it can be accurately transferred to the test tube. At such a time, if the gauze inadvertently touches the tip of the pipet, the sample in the pipet will flow into the gauze rendering the sample too small to measure. Therefore, before transfering the sample to the test tube make it a practice to check the other end of the pipet through the transparent wall of the dome after wiping with the gauze to determine if the pipet is completely full with the sample and there is no shortage.

3) The tip of the pipet is placed against the wall of a clean small test tube into which the sample is to be transferred, the hole of the dome is covered with the finger and pressure is applied to the bottle by squeezing the thumb and middle finger. All of the liquid in the pipet will be transferred to the test tube, thus an aliquot of the sample equivalent to the volume of the pipet will have been measured into the test tube. The pipet should be removed from the test tube by gently drawing the tip up along the wall.

Pipetting by use of a Sanz's pipet is extremely simple and even an inexperienced technician can master its use for a short period of practice. According to Saito,[14] on the day that he used the Sanz's pipet for the first time, his error rate in pipetting 100λ was 1.4 % (CV : coefficient of variation), which dropped to 0.9 % one week's practising and decreased further to 0.4 % after one month. At the end of one month it can be operated at 1/2 the measurement error of the traditional Mohr ultramicro pipet procedure.

In the authors' experience in measuring 20λ, the error rate has never exceeded 0.5 %. Further, with only 1 or 2 days' practice one can attain this degree of accuracy. When handling 50 to 60 samples in succession, it is possible to measure 1 sample at the average time of 10 seconds. This is a good rate that equals sample measuring under the micro method. As it is not necessary to match the liquid level with a mark at the time of measuring, the procedure can be carried out free of such concern. Furthermore, as the pipet is made of polyethylene, there is no fear of breaking it as in the case of glass.

The 20λ pipet is most easy to use. Therefore, the authors unified their system so that ultramicro determination is performed, as much as possible, with a 20λ sample.

When a Sanz's pipet is used, it is possible to pipet directly serum which has separated in a capillary tube. With the conventional ultramicro pipets, in order to collect the serum, it was necessary to cut with a file the capillary tube at a place slightly above the line of serum-and-clot. However, as the tip of the Sanz's pipet is small and rather flexible, it is possible to insert it directly deep into the serum layer in the capillary tube to aspirate the serum* (Fig. 9–2)).

Washing the Sanz Pipet

Prior to using the Sanz pipet, always draw in and expel H_2O a few times. At such time, if an air bubble should form within p, it is a sign that the pipet is not clean. Washing should be conducted as follows :

a) To remove simple contaminants :

Draw neutral detergent (1～2 g/dl solution of Wonderful K etc.) into the pipet

*A file (or ampule cutter) mark is made on the glass capillary tube along the upper portion of the serum layer and the tube is snapped in two at this section. The unneeded glass portion of the tube is discarded making it easier to make collection with the Sanz's pipet.

and let it stand for 5 minutes. This solution is then drawn into the bottle and the pipet is washed out 4–5 times with H_2O. H_2O is drawn into the pipet once again to determine whether any bubbles will develop. If bubbles continue to appear in the pipet, it should be washed out with concentrated ammonia solution.

The pipet should be filled with concentrated ammonia solution, left to stand for about 15 minutes and then washed out 4–5 times with H_2O. Check is made to determine if bubbles will appear. Generally, none should develop after such processing.

Actoene is drawn into the pipet followed by air to dry the bore. Next, the dome (D) is removed from the bottle (B) and the interior of the bottle is washed out repeatedly with H_2O*. The dome is replaced and the apparatus is ready for measurement.

After pipetting serum with a Sanz pipet, always wash its interior by drawing water through the pipet a few times. This will prevent serum from adhering to the inner surface of the pipet and obstructing the bore.

b) When the pipet is obstructed by serum protein coagulation:

If, by accident, serum protein should adhere to the inner cavity and become coagulated resulting in obstruction of the pipet, it will be impossible to remove this by the procedure a) using neutral detergent and ammonia. Even immersion of the pipet in digestive ferment (e.g. pepsin) will not reopen the bore**.

One will become tempted to squeeze the pipet with the tips of his fingernails, but this should be strictly avoided. The pipet will be flattened and the volume will become inaccurate. Further, any damage or deformation of the inner cavity of the pipet will create a tendency that a part of the sample to remain in the bore, rendering accurate pipetting impossible.

The best method would be to slowly insert a fine mandrin (stylet) into the pipet from the tip once (only once) and make a hole in the clot of protein so as to allow the passage of air. Attempts should not be made to scrape it free with the mandrin. Then, draw in concentrated ammonia and let it stand for about 15 minutes. The serum protein will be dissolved and the pipet will be reopened.

c) When the pipet is contaminated by reagent:

When the pipet is contaminated by reagent which cannot be removed by concentrated ammonia, dichromate sulfuric acid or hydrochloric acid is drawn into the pipet and allowed to stand for 15–30 minutes. This should remove all contamination. The pipet should be washed several times with water and dried

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*Never neglect washing out the bottle. If there is any ammonia or acetone remaining in the bottle, they will become mixed with the sample and disturb quantitation.

**As neither neutral detergents nor ammonia can enter the pipet, they cannot get into contact with the protein which is obstructing the bore. This is the reason why they are not effective in such an event.

by passing acetone through it.

d) A convenient Sanz pipet washing set to keep on hand:

Obtain bottles similar to those used to hold standard solutions for calibration curves (empty Eiken Disk bottles), fill one with neutral detergent, the next with concentrated ammonia, another with acetone and the last with H_2O, respectively, and place on a bottle stand. Also, have available mandrins. These should be arranged on a table and made available as a washing set. The H_2O should be changed daily.

3. Pipetting the reagents—Minipette[15]*

In ultramicro determinations usually 0.2–2.0 ml of reagent is added to the sample (20λ) to produce coloration. It is quite troublesome to measure reagents in volumes less than 1.0 ml with the ordinary measuring pipet which is employed in micro-analysis. Furthermore, when making serial pipetting with such measuring pipets, there is an average error of 3 %. If micro-analysis procedures with the mere use of finer graduated pipets are adopted in ultramicro method, determinations will require great mental strain making it impossible to process a large number of samples. It is felt that the greatest reason why ultramicro methods have not been widely accepted is that pipetting procedures suitable for ultramicro determinations have not been developed. The measuring procedure using a minipette to be introduced below has not only overcome this difficulty, but has contributed greatly to making the ultramicro method into a procedure more pleasant and efficient than the micro method.

Principles of the Minipette

The principle is similar to a piston type pump where a fixed volume of liquid is drawn in and expelled by the upward and downward movement of the piston. Further, by use of an adjusting screw the range of piston travel can be changed, thus making it possible to adjust the liquid volume at will. Naturally, it will be necessary to have a pressure device. For this purpose a device which has a cylinder (or barrel) fitted with a plunger that rotates together around a cock was made. When the cylinder and plunger are placed down in the "lower position", the plunger will retreat by its own weight and liquid will be drawn into the cylinder, and when they are brought up to the "upright position", the plunger will apply pressure upon the liquid in the cylinder forcing it out. This is the Minipette. By special designing of the cock, it is possible to automatically and successively switch the connection from the cylinder to the reagent container or the introducing pipe during low and upright rotations of the position.

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*Manufacturing and sales are handled by Kayagaki Irika Co., Ltd. (14–16 Hongo 3-chome, Bunkyo-ku, Tokyo).

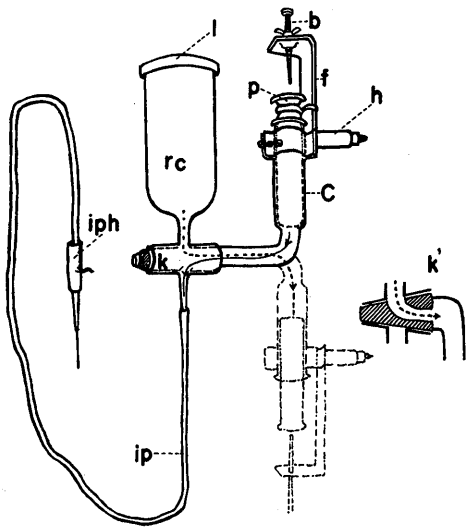

Fig. 10. Structure and principles of Minipette

b : Adjusting screw; c : Cylinder; f : Frame for holding cylinder; h : Handle; ip : Introducing pipe; iph : Introducing pipe holder; k : synchronous cock; k' : Cross section of synchronous cock (A hole runs through the central axis of the cock. Therefore, when the cock is rotated, c and rc become connected, and it is likewise possible to connect c and ip); l : Lid; p : Plunger and rc : Reagent container.

The cylinder is rotated around cock (K) by the handle and brought to the position indicated by dotted line. In this position, reagent will flow from reagent container (rc) to cylinder (c) as shown by the dotted arrow line, and when returned to the position shown in full line, the reagent will flow to introducing pipe (ip) from cylinder c as indicated by the full arrow line.

The structure of the Minipette

The structure is as shown in Figure 10. The names of the various parts are given below :

ap : Aspirating pipe (a polyethylene tube with an inner diameter of 3 mm).
b : Liquid volume adjusting screw
c : Cylinder or barrel (a tuberculin syringe...1-2 ml...or a 5 ml syringe*).
f : Frame for holding the cylinder
g : Rubber stopper (No. 14).
h : Handle
ip : Introducing pipe (a polyethylene tube with an inner diameter of 3 mm).
iph : Introducing pipe holder
k : Synchronous cock (When the handle is in the upper position, cylinder c and introducing pipe ip will be connected, and when it is rotated to the

*The size is determined by the volume of the cylinder. Those of 1 ml, 2 ml and 5 ml in volume are called No. 2 and No. 5 syringes,respectively.

Preparation

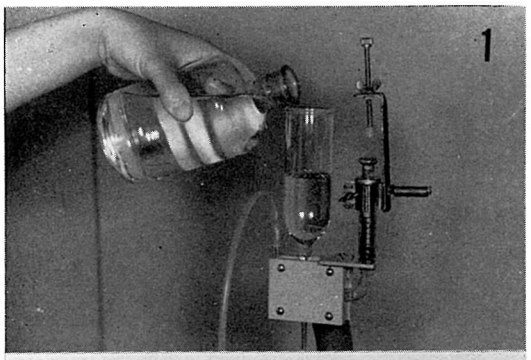

Photo 1.

Preparation 1: Reagent is poured into the reagent container.

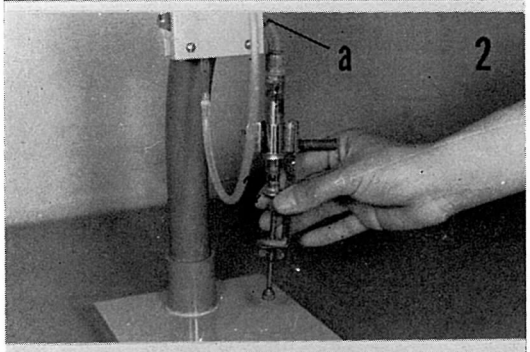

Photo 2.

Preparation 2: Eliminate all bubbles in the cylinder.
Bubbles (a) can be forced out by moving the plunger up and down.

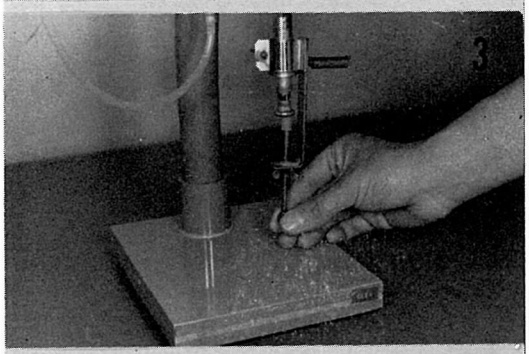

Photo 3.

Preparation 3: The adjusting screw is fixed after the reagent volume has been determined.

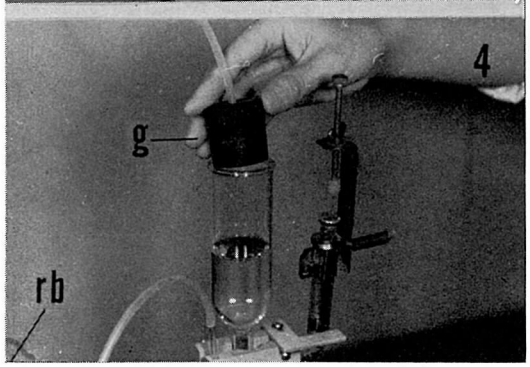

Photo 4.
Preparation 4: Rubber plug (g) is inserted into the reagent container and the tube is connected to reagent bottle (rb) so that reagent can be continuously and automatically supplemented.

lower side, the cylinder and reagent container rc will be connected.).

l : Lid

p : Piston (or plunger)

rp : Reagent bottle (Capacity about 500 ml)

rc : Reagent container (Capacity about 100 ml, diameter about 4 cm)

t : test tube, sizes 10 × 70 mm (No. 1), 12 × 70 mm (No. 2) or 15 × 70 mm (No. 3)

(ap, g, rb and t are not shown in the Figure)

Preparation of the Minipette

(1) Remove lid (1) and put reagent into reagent container (rc). **(Photo 1)**

(2) Rotate handle (h) to lower position, take the end of plunger (p) between the fingers and slowly pull downward. Reagent will fill the cylinder (c) and all bubbles will be forced out into reagent container (rc). **(Photo 2)**

(3) If there are any bubbles at the upper end of introducing pipe (ip), bring handle (h) to the upright position and pull plunger (p) up. The bubbles will be drawn into the cylinder through synchronous cock (K). They can then be driven out by (2) above.

(4) Liquid volume adjusting screw (b) is turned until the end of the plunger is in level with the required graduation on cylinder (c). Thus, the range of travel is set and the fixing screw is tightened. **(Photo 3)**

(5) When a large volume of reagent must be delivered in serial doses, rubber plug (g) of aspirating pipe (ap) should be fitted into the mouth of reagent container (rc) and sealed, and the tip of aspirating pipe (ap) should be inserted into reagent bottle (rb)*. This will make it possible to supply reagent container from the reagent bottle through the aspirator pipe each time the handle goes through an up and down rotating motion. **(Photo 4)**

Operating the Minipette

(1) Test tubes (t) are put in the test tube stand which is placed near the apparatus. Handle (h) is taken in the right hand and the tip of introducing pipe (ip) is held in the left hand with the tip inserted into a test tube. **(Photo 5)**

(2) Handle (h) held in the right hand is tilted forward. (If it is tilted toward the operator, it will hit the test tube stand and make operations inconvenient.) **(Photo 6)**

(3) Handle (h) is rotated to the lower position and check is made to determine whether the tip of plunger (p) comes into full contact adjusting screw (b)**. **(Photo 7)**

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*Fill reagent container (rc) to 2/3 with reagent, perform procedures (2)-(4) and then insert plug (g). This is a point to take note of.

**When the operator becomes familiar with the operations of the Minipette, he will be able to tell that the plunger has fully retreated by the "click" sound made when the tip of the pipet and adjusting screw (b) come into contact with each other.

Operation

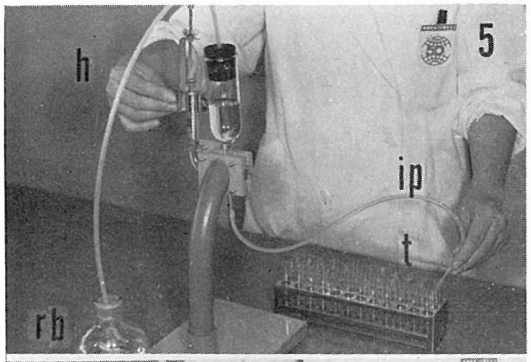


Photo 5.

Operation 1 : Take hold of handle (h).

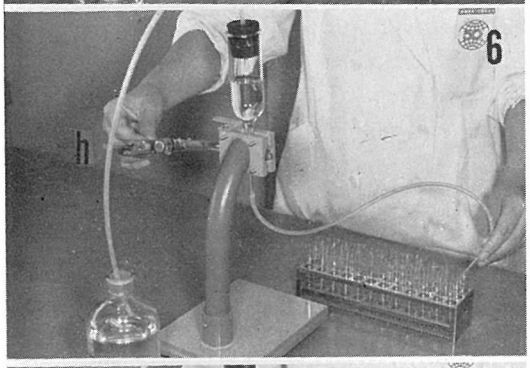
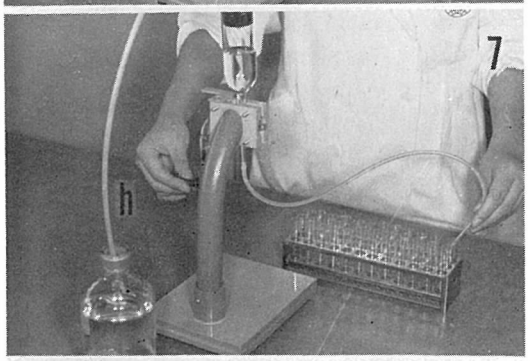


Photo 6.

Operation 2 : Rotate forward (away from operator).



Phoro 7.

Operation 3 : Stop handle (h) at lower position (tip of plunger will be in contact with tip of adjusting screw).

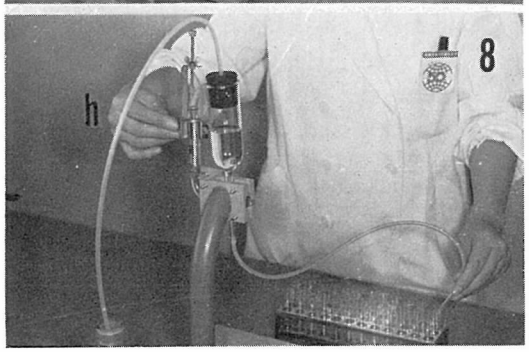


Photo 8.

Operation 4 : Bring handle (h) back to upward position (liquid will be sent from the Minipette to test tube (t)).

(4) After handle (h) has been returned to upright position*, the plunger will retreat under its own weight** forcing a predetermined amount of reagent (determined by adjusting screw setting) into test tube (t). **(Photo 8)**

(5) Put the tip of introducing pipe (ip) into each of the new test tubes and repeat procedures (1)–(4). Thus, serial transfer of a fixed amount of reagent to test tubes can be accomplished.

Time required for measuring with a Minipette

Reagents may be measured with great speed. Serial measurement of 0.2 ml of reagent requires only an average of 2 seconds per measurement and 4 seconds for the measurement of 2 ml.

Precision of Minipette

Variation of the amounts (weight in mg) of a reagent solution of a certain fixed volume taken by successive operation of a minipette is shown in Table I. It is seen from this table that the maximum error is less than 1 %, less than 0.2 % and less than 0.1 % for the pipetted volumes of 0.1 ml, 1.0 ml and 3.0 ml, respectively.

Table 1. Precision of Minipettes

Magnitude of measurement	Measurements by Minipette					
	0.1 ml		1.0 ml		3.0 ml	
	Wt. of water measured (mg)	Variation from the smallest value (mg)	Wt. of water measured (mg)	Variation from the smallest value (mg)	Wt. of water measured (mg)	Variation from the smallest value (mg)
1	106.7	+0.9	1,048.8	+0.8	3,044.5	+0.1
2	106.8	+1.0	1,050.8	+2.0	3,045.9	+1.5
3	106.5	+0.7	1,048.9	+9.0	3,046.4	+2.0
4	106.5	+0.7	1,049.3	+1.3	3,046.3	+1.9
5	106.2	+0.4	1,048.4	+0.4	3,044.4	0
6	105.9	+0.1	1,049.7	+1.7	3,045.5	+1.1
7	106.3	+0.5	1,048.9	+0.9	3,045.6	+1.2
8	105.8	0	1,048.0	0	3,047.4	+3.0
9	106.0	+0.2	1,048.3	+0.3	3,047.5	+3.1
10	106.1	+0.3	1,049.0	+1.0	3,046.7	+2.3
Maximum difference	less than 1 %		less than 0.2 %		less than 0.1 %	

The maximum difference refers to the difference between the largest and the smallest weight (observed in 10 consecutive measurements) divided by the smallest weight, which is expressed in terms of percentage.

*It is important to bring the cylinder into perpendicular position.

**This can be determined by the "click" sound when the tip of the plunger strikes the lower end (bottom) of the cylinder.

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