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DEPARTMENT OF THE ARMY
Fort Detrick
Frederick, Maryland
PREVENTION OF LABORATORY INFECTIONS

[Following is translation of a German-language communication by Ferdinand Reinhardt, MD, Austrian Army Physician, in Zentralblatt für Bakteriologie und Parasitologie (Central Journal for Bacteriology and Parasitology), Vol. 80, 1918, No. 7, pp. 456-465.]

The measurement of fluid quantities in fractions of a cubic centimeter is a daily necessity for the bacteriologist and the serologist. Up to the present time, this is done in most cases still by means of the customary oral pipette. Bacteriologists have been aware from the beginning that this method, especially when dealing with bacterial suspensions, represents considerable dangers and only the skill and attention of the operator prevents the fluid from entering the mouth. Kisskalt (1) has published a compendium of laboratory infections from typhus based on a questionnaire from which it results that one-half of 57 cases -- as assumed by Paneth (2) -- and possibly a greater number must be blamed on this pipette method. Obviously, both physicians and designers have endeavored to invent mechanical devices eliminating the necessary suction by mouth.

The simplest arrangement for this purpose is probably the familiar eye dropper; a customary glass pipette is provided with a small narrow rubber bulb at one end which serves to draw in and expel the fluid. Ref. 2 recently contained a recommendation for this rubber-bulb pipette as designed by Wright (20) and indicates a proper position of the fingers for its manipulation.

Kitt (3,4) has expanded the pipette at the top in the shape of a funnel and provides airtight closure by means of a rubber membrane. Pressure with the index on this membrane has the same effect in a perhaps more convenient manner than with a rubber bulb. The same author (5) also indicates a special form of the rubber bulb which has the shape of a harmonica and is equipped with a ring for the index so that the bulb can be easily distended so as to furnish the desired suction.

- 1 -
Recently, Neumayer (6) has devised a rubber bulb for pipettes which is being manufactured in two sizes for 1-ccm and 10-ccm pipettes. The bulb possesses a reinforced rubber tube for insertion of the pipette which runs through the entire bulb and has special openings for circulation and slots in the interior for easy disinfection.

A modification of the bulb pipette by Permin (7) consists in passing an elongated rubber bulb with two opposed openings over the pipette tube so that the top of the glass tube extends above the bulb. Through a small opening in the wall of the glass tube, the interior of the bulb communicates with the interior of the pipette. If the bulb is compressed and the tube closed at the top with the index, fluid rises in the pipette when the bulb is released. When the index is lifted, the content flows out to the desired amount. The advantage of this arrangement consists in the fact that the flow of the fluid drawn in is released, as in the oral pipette, by lifting the index. Instead of a specially formed bulb, it is frequent enough to find in practice merely a piece of rubber tubing which is closed at one end in some manner and is similarly servicable. Instead of rubber suction devices, we recently find more frequently little suction devices which consist of a cylinder cemented to the pipette tube in which a piston is automatically returned to the starting position by a coil spring after it has been activated.

With the aid of the devices described, it is possible to work quickly and more quickly than with the oral pipette; the apparatus is very simple, can generally be operated with one hand and if it becomes necessary, as for e.g. agglutination, to fill a number of tubes with 1.0 or 0.5 ccm of a fluid, accuracy is still sufficient, even if we take into account a small number of lost drops. However, if it becomes necessary to draw about 0.1 or perhaps only 0.05 ccm of the serum for dilution from above the blood clot, accurate and exact measurement of these small quantities is difficult even with the necessary attention and skill since the inevitable small unsteadiness of the fingers causes the fluid in the necessarily narrow pipette tube to continually move up and down, occasionally to flow or to drip out, especially if visual control is eliminated, e.g. when setting the glass tube aside. We must then repeat the process if we still have enough serum or if the blood corpuscles have not already returned into suspension after the first try. Many investigators prefer the oral pipette method but interpose rubber tubing with a glass mouthpiece which is retained in the mouth during the entire pipetting process. This permits rapid and reliable working under visual control but it should be possible to eliminate the aid of the mouth completely in the bacteriological laboratory.

Such tubing may even form the basis for a precision suction device if it is arranged so that it can be compressed between two rollers for any desired length without having to utilize suction by mouth or pressure with the fingers. Such a device, with an appropriate positioning of the rollers which can be easily rotated with the thumb so that the instrument
A simple pipette suction device on the principle of the Strohschein syringe can be produced if we slip a suitably narrow test tube over a customary glass pipette and a short piece of rubber tubing over the smooth edge of the test tube which slightly projects above the edge of the latter and consequently surrounds also the pipette, obviously less firmly, but still airtight. By sliding the two glass tubes against each other, it is obviously possible to draw fluid into the pipette through the vacuum thus created. Such a poison pipette devised by Meyer (9) has an opening at the top of the outer glass tube which must be closed with the finger for suction but allows any desired amount of the content to flow out when the finger is lifted; Wassermann (10) recommends placing the air hole not at the top but more conveniently on the side. The suction pipette of Wolff (11) is designed on the same principle. When drawing up fluids with these devices, both hands are required as rule.

A large number of devices for the purpose are based in principle on the fact that, in the cylinder of a syringe, an airtight closure can be adjusted by means of a micrometer screw which will cause fluid to rise into the pipette. Such devices are justifiably called precision suction devices or micropipettes because they permit accurate and reliable measurement of even the smallest quantities of fluid. In addition to these advantages, it is regarded almost without exception as a disadvantage that both hands are required for drawing in and expelling the fluid; the left hand for holding the instrument and the right hand for adjusting the plunger (rotation of the screw). Moreover, exchange of the pipettes which often must have a definitely given shape and size as well as the frequent rotation of the screw with amounts of about 1 ccm very often absorbs a considerable amount of time. This last factor was taken into account several years ago by the Leitz Company by utilizing instead of an ordinary thread a steep screw (worm pitch). There is no doubt that these deficiencies have been the cause that these otherwise satisfactory instruments have not been generally adopted. In many cases, however, they are used in particular for blood-corpuscle pipettes, e.g. the small precision suction device of Wieck. Among those belongs also the modification of Brandt (12) which utilizes the screw disc of the plunger stem for adjusting the plunger of a Pravaz syringe of an old model with hard-rubber mounting.

Here we should also mention the precision suction device of Hoerder (13) which is distinguished in particular from the familiar designs by having the entire device mounted on a support so that the hands are left free. As in the models referred to above, here also the plunger is adjusted by an accurate micrometer screw, and a completely airtight closure of the plunger is accomplished in an ingenious manner with mercury. The capillaries or pipettes are connected by
means of rubber tubing. The author recommends the suction device in particular for determination of the opsonic index; for ordinary work with greater quantities of fluid of about 1 ccm, the device is less useful.

Miller (14) has perhaps solved the practical side best with his design. It consists of a low but wide cylinder which is divided by an elastic membrane. A metal disc presses on the membrane and this disc can be moved up and down by a screw passing through the top of the cylinder. The cylinder is closed at the bottom by a rubber stopper with a central bore in which the capillaries are inserted. The essentially new feature is a pistol-grip projection on the side of the cylinder wall which is firmly gripped in the hand during use while the thumb and eventually the index are left free for activating the screw. This suction device is thus operated with only one hand, permits accurate measurement of very small quantities of fluid, and the exchange of the pipettes is made rapidly by merely inserting them. The device is a micropipette and also specifically designed for opsonin determination. Measurement of larger amounts of fluid of about 1 ccm would require more frequent rotation of the screw and consequently an undesirable loss of time.

In order to increase working speed, it seemed obvious to operate the plunger directly instead of by means of a screw. Accordingly, the precision suction device of Woithe (15) essentially consists of a 2-ccm metal syringe in which the piston is moved directly with the thumb. For this purpose, the piston stem has a ring for the thumb and the cylinder two lateral rings for the index and third finger. The free end of the cylinder is continued into a tube which is bent in the shape of a U and has a clamp for airtight insertion of the pipettes. The cylinder further has two spring forks by which the pipette is firmly held in position. Through the opposed orientation of the pipette, the entire device has been shortened in an ingenious manner and has a much higher degree of maniability; it is operated with one hand.

However, the change of pipettes requires several manipulations: 1. insertion of the pipette in the spring forks; 2. insertion in the clamp; 3. tightening of a holding nut; the pipette is removed with the same manipulations in reverse order. Moreover, the position of the fingers in holding the device creates a lateral pressure on the plunger stem and consequently a tendency of the plunger to jam very often at the decisive instant which tends to produce dissatisfaction with the working of the device.

The safety suction device of Koch (16) also consists of a cylinder with an airtight metal plunger which can be displaced by means of a knob projecting through a slit in the cylinder in the manner of a pocket pencil. The instrument is operated with one hand. The pipettes are mounted with rubber tubing and inserted with this in the receiver of the instrument for which the pipettes must be suitably formed at their upper end.
Very ingenious is the micropipette of Weichardt (17) which is specifically intended to measure small amounts of fluid quantitatively accurately, rapidly and repeatedly. An accurately calibrated glass tube which is tapered at both ends is completely passed through a rubber stopper which forms the closure of the cylinder of a syringe so that the upper end of the tube enters some distance freely into the cylinder space. If the syringe plunger is raised, the tube becomes filled with fluid and an excess collects in the cylinder space. Consequently, the device belongs among the group of overflow pipettes and has the advantage that the plunger rise does not need to be adjusted carefully and always exactly the same amount is expelled when pressing down on the plunger. With an appropriate construction, the instrument would be suitable for one-handed operation; however, it requires special pipettes and exchange of pipettes when changing to another amount of fluid.

Another pipetting method consists essentially in manipulating the pipette in the manner of a thief tube, i.e. it is immersed in the fluid as far as the desired gradation, closed by a finger at the top and withdrawn containing the desired amount.

In order to be able to remove the fluid except for a minor rest, Reiner-Müller (18) has indicated a "tulip-shaped pipette tube" which Wagner (19) has recently recommended as agglutination tulip. However, it should be pointed out that with this working method the pipette collects fluid on the outside; immediately after removal, about 6-12 drops fall from the surface in the first 5-10 seconds, depending on the size of the pipette. We thus have to wait during this interval if we want to prevent that any drops of the possibly infectious fluid drop anywhere on the way to the test tube. Otherwise, working in this manner is very convenient and causes no difficulty in changing pipettes. In regard to speed of operation, this method is surpassed by a great many of the devices mentioned earlier; moreover, it is not suitable for the removal of serum by pipette. According to a communication by Wagner, the Institute at Kiel carries out the measurement of small amounts of serum in 1-ccm "Record" syringes which are equipped with extra long cannules for this purpose and are suitably cleaned after each use.

In the foregoing, I have attempted to briefly describe the better known pipette devices as far as they are suitable for bacteriological purposes and to indicate their advantages and disadvantages. It is possible that, in addition to those devices named here of which I know the greater part from personal knowledge, there are other modifications which would doubtlessly fit without difficulty in one of the groups described.

It is evident that a good deal of ingenuity has gone into designing generally useful pipette suction devices with the result that even in the foremost bacteriological institutes the oral pipette continues to be used; at the most, the rubber bulb or one or the other
device for special purposes has found a certain degree of adoption in individual institutes.

After having occupied myself with this problem for years and without success, I am now able to indicate a pipette suction device and its manipulation which has been highly satisfactory in almost three years of practice and in particular under the difficult circumstances in military field hospitals.

The device (registered) consists in general of a syringe in which the plunger can be moved directly up and down with the index because a specially adjusted plunger and a suitable finger hold are provided on the plunger stem for this purpose. Movement of the plunger creates a vacuum or air pressure in the cylinder of the syringe so that fluid can be drawn into and expelled from a pipette which is connected to it by an airtight intermediate connection (fig. 1). A graduated glass cylinder, capacity 3 ccm, is provided with a long solid metal plunger which fits almost airtight into the cylinder. The plunger stem projects through a screw cover and carries at the outer end a suitably formed collapsible finger hold on a cross piece. The pistons of the finger hold fit into corresponding holes of the cross piece with sufficient friction so that the former can be rotated but does not drop under its own weight.

The plunger has a groove in the center of its circumference which contains a spring. The latter is not intended to make the plunger airtight but to provide sufficient friction against the barrel of the syringe to maintain the plunger in any desired position. The barrel terminates in a tapered metal end piece with a central bore to which an intermediate connection can be attached airtight.

The intermediate connection has a metal rim for convenient manipulation and terminates in a thin tube (fig. 2) or in a hollow needle to which a suitably shaped cork is fitted which can be rotated. The cork has, at the upper end, the same diameter as the metal rim; the lower end is thin and tapers slightly so that it can be inserted in the particular pipette utilized.

For particularly small or thin pipettes or capillaries, another intermediate connection has been designed (fig. 3) which carries a rubber stopper with a central bore instead of the cork and here the pipette is inserted into the rubber stopper. This type of intermediate connection with bores of different diameter in the rubber stopper can be utilized for any type of pipette and has the advantage that the pipette is connected flexibly with the instrument which may be preferred by many investigators.

Prior to first using the instrument, it is necessary to coat the latter on the inside with some lubricant, e.g. yellow vaseline,
if this has not been done previously. This lubrication is essential and has as result on the one hand a completely airtight closure of the plunger of piston and, on the hand, provides for slow uniform movement of the plunger. When so prepared, a weight of 70–100 g attached to the finger hold should compensate the friction of the plunger spring and cause the plunger to descend over the whole length of the barrel within about 16–20 sec. For smooth operation, this adjustment has proved to be best.

During use, the instrument is held between thumb and the third and fourth finger at the rifled metal top and the index is inserted in the finger hold for activating the plunger (fig. 4). The hold must have the proper size and form so that the first joint of the finger does not slide all the way through the hold.

Subsequently, the pipette is fitted with the intermediate connection and is attached at the end to the syringe with a slight rotation, made conveniently possible by the metal rim of the intermediate connection. When necessary, the gradation of the pipette is turned toward the light, so that it will be easily readable, by turning the pipette with the cork around the hollow needle. When utilizing the rubber stopper, the gradation of the pipette must be properly placed at the time it is inserted but it is also possible to rotate the entire instrument around the plunger with the thumb and third finger of the right hand alone.

If we now immerse the lower end of the pipette in a fluid and raise the plunger with the index, it is surprisingly easy and accurate to draw the fluid up to the desired gradation. The fluid remains firmly in the pipette and can be expelled by pressure on the plunger. If the fluid should leak, the only reason for this will be a defective cork. Suitable corks from an appropriately homogeneous piece can be prepared by anybody with a slight degree of skill by using a sharp knife and sandpaper. A leaky fit of the intermediate connections is very seldom the case and can be corrected by an extra twist against the tapered metal end. To prevent the prepared pipette from becoming disengaged from the syringe taper, we recommend an energetic rotation, eventually by utilizing chalk, when connecting the two components.

As described, the design completely corresponds to the requirements for an efficient pipetting device and has the following advantages:

1) the device is operated with one hand;

2) the manipulation is convenient and exactly the same to which we are accustomed with usual methods of pipetting;

3) even very small amounts of fluid can be drawn in and expelled rapidly, reliably and quantitatively accurately under visual control;
4) the fluid column drawn into the pipette is firmly held in place;
5) pipettes can be exchanged rapidly by simple insertion;
6) any desired pipette up to 2 ccm maximum can be utilized;
7) in special cases, it is possible to pipette with adequate accuracy even if the pipette is not graduated;
8) rapidity of manipulation with this instrument is superior to all other methods;
9) if the instrument becomes contaminated, it can easily be taken apart and sterilized by boiling;
10) the simple design is failure-proof and has an indefinitely useful life when treated properly.

It should be pointed out that this suction device is suitable for use with any pipette up to a maximum of 2 ccm, provided a suitable intermediate connection is available or prepared. However, we recommend pipettes as short as possible (17 cm maximum) and as light as possible which make working more convenient and also more agreeable. The frequently utilized oral pipettes for 1 ccm of thick glass with a length of 30 cm are less suitable because of their size and weight.

In emergencies or in cases where absolute accuracy is not required, the device makes it possible to pipette sufficiently accurately with any given glass tube by reading the gradations on the glass barrel of the instrument which are provided for this purpose. This method will be especially suitable for the measurement of stain solutions, removal of sediment from deep vessels, drawing of infectious provocative serum from experimental animals, etc.

In order to reach maximum operating speed with this pipette device, it will be necessary to describe in detail the method of operation through examples and to point out some individual shortcuts.

For example, we may need to carry out, for a number of blood specimens, the Gruber-Widal reaction for typhus in serum dilutions of 1 : 50, 1 : 100, 1 : 200, etc.

We first prepare for each blood specimen a number of agglutination tubes and provide two 1-ccm pipettes and one 0.5-ccm serum pipette with the appropriate intermediate connections. With one of the former, we measure into the first tube of each series 0.96 ccm NaCl (physiological salt solution) and in each of the other tubes 0.5 ccm of the solution.
For the subsequent removal of the serum from above the blood clot, we recommend a very thin 0.5-ccm serum pipette in which the gradations of 1/100 ccm obviously are spaced twice as wide as in the 1-ccm pipette of the same length and thus permit more accurate measurement.

With this, we draw off accurately 0.04 ccm of serum and transfer it to the first tube. Although we have the pipette device in the right hand, it is still possible with the fourth and fifth finger of this hand to remove and replace the stopper of the blood-specimen tube. Stoppers which stick to coagulated blood must be loosened beforehand.

In order to completely expel the serum from the pipette, it is necessary to always slightly raise the plunge before use so that the barrel will contain sufficient air for the purpose of blowing out the pipette after expulsion of the measured content.

With 2-3 subsequent brief plunger movements and by holding the point of the pipette in the fluid close to the surface, sufficient mixing is accomplished as will be seen from the disappearance of the streaks. However, we should be careful not to draw the mixture beyond the pipette into the instrument itself.

From the first tube which contains a serum dilution of 1 : 25, we transfer 0.5 ccm to the second tube, mix it, transfer 0.5 ccm to the third tube, etc. and one-half of the last tube is discarded.

The pipette is then flushed out with physiological salt solution or water one or two times. At the point of the pipette, a small amount of flushing solution collects which must be removed before proceeding with measurement. For this, the pipette and the intermediate connection together are removed and the point is brought into contact with a pad moistened with mercury chloride — in a Petri dish — which immediately absorbs any remaining solution. At the same time, the plunger is slightly raised to provide an excess air volume in the barrel for blowing out the syringe. The pipette then is ready to work on the second blood specimen and the preparation of further dilutions. Immediately before insertion into the serum, additional fluid will again have collected in the point of the capillary (about 0.005 ccm) and is again removed through contact with the pad and slight pressure on the plunger.

We thus use — horribile dictu — one and the same serum pipette for the preparation of dilutions of all specimens. However, we should here point out that the blood specimens themselves are frequently not sterile, that absolute sterility is not necessary for agglutination tests — agglutinines can be demonstrated even in decomposing blood — that frequently carbolic-acid suspensions are utilized resulting in growth inhibition of possibly existing germs and that finally the risk of transferring agglutinines from one serum to the next in a demonstrable quantity does not exist in reality for the described method, even then when a high-titer serum is followed by a completely negative serum.
Let us assume we proceed without flushing and continue to use a tube containing e.g. a dilution of 1:200 for the next serum while a residue of 0.01 ccm remains in the pipette. If we do not remove this residue, it becomes diluted 100 times in the subsequent 1 ccm which results in a dilution of 1:200 x 100 and, when we add the same quantity of germ suspension, a final dilution of 1:40,000 which is already more than the titer of most test sera.

The serum dilutions have been prepared in the tubes and we now add, with a third pipette, 0.5 ccm of the germ suspension to each one— which automatically produces adequate mixing—so that we obtain the desired dilutions 1:50, 1:100, 1:200, etc.

While filling the tubes with physiological salt solution and the germ suspension, they are best left in their holders; however, in the preparation of the serum dilutions, each tube must obviously be held in the left hand.

Let us point out once more that, during operation, the pipettes must always be attached and removed together with the intermediate piece which saves time and the cork and/or rubber, and there is practically no wear on the metal taper. The attached table shows the magnitude of error when the operator does not work sufficiently accurately.

The table shows that, when drawing off the serum, a plus or minus above one-half of one gradation = 0.005 ccm already constitutes an error of more than +/- 10% whereas, when measuring the physiological salt solution, an inaccuracy of one gradation results in an error of only +/- 1%. The error will be as much larger as less serum is utilized. Anyone not confident of being able reliably to measure 0.04 of serum, should use twice the dilution with 1.92 NaCl and 0.08 of serum or else utilize an auxiliary tube at about 1:5.

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<th>(b) Erzielte Endverdünnung</th>
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<tr>
<td>0.04 Serum + 0.96 NaCl</td>
<td>1:25 1:50 1:100 1:200 1:400 1:800 1:1600</td>
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<tr>
<td>0.06 Serum</td>
<td>1:33 1:66 1:132 1:264 1:528 1:1056 1:2112 1:4224 1:8448 1:16896</td>
<td>32%</td>
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<tr>
<td>0.06 Serum + 0.96 NaCl</td>
<td>1:29.2 1:48 1:96 1:192 1:384 1:768 1:1536 1:3072 1:6144 1:12288</td>
<td>90%</td>
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<tr>
<td>0.04 Serum</td>
<td>1:22.2 1:44 1:88 1:176 1:352 1:704 1:1408 1:2816 1:5632 1:11264</td>
<td>40%</td>
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<tr>
<td>0.04 Serum + 0.96 NaCl</td>
<td>1:24.75 1:49.5 1:99 1:198 1:396 1:792 1:1584 1:3168 1:6336 1:12672</td>
<td>10%</td>
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<tr>
<td>0.04 Serum + 0.97 NaCl</td>
<td>1:25.25 1:50.5 1:101 1:202 1:404 1:808 1:1616 1:3232 1:6464 1:12928</td>
<td>8%</td>
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(a) = starting dilution; (b) = terminal dilution; (c) = error in %.

If 10 blood specimens are to be processed in the manner described and each specimen tested in 10 dilutions, i.e. a total of 100 tubes,
then the entire work of introducing physiological salt solution, preparation of serum dilution and completion with germ suspensions can be carried out in 22 min, i.e. a speed which can not be reached with the same accuracy by any other pipetting method.

The foregoing was intended primarily to explain the typical method of operation with this pipetting device.

During mass testing, especially at times of different typhoid diseases, we have found very useful for preliminary orientation e.g. the following agglutination series: for typhus and paratyphus B as the most frequently agglutinized strains, dilutions of 1:50 and 1:100; for paratyphus A and for a strain particularly involved at the time and in the locality, e.g. Weil-Felix proteus for typhus-fever diagnosis, a dilution of 1:50. A dilution below 1:50 is useless for diagnostic purposes.

Accordingly and corresponding to the number of holes in the customary agglutination holders, 6 tubes are needed for each blood specimen. By this we mean the customary agglutination tubes, 60 cm long and 10 mm in dia. with a maximum capacity of 3 ccm.

In the first tube, we prepare a dilution of 1:25 with 2.4 ccm NaCl and 0.1 serum and distribute 0.5 ccm each for paratyphus A, paratyphus B, and x into the given tube for a dilution of 1:50; 0.5 ccm remains in the first tube for typhus 1:50 whereas the last 0.5 ccm in the second tube is mixed with 0.5-ccm NaCl already prepared and this dilution is halved for typhus and paratyphus B 1:100. Consequently, we find in each of the 6 tubes 0.5 ccm of serum dilution which produce the given terminal dilutions when mixed with the same amount of the respective germ suspension. 10 blood specimens can be processed within 20 min by this method. With positive findings from this orientation series which in practice sufficiently frequently permits recognition of the terminal titers, the dilutions for one or the other strain are continued to the terminal titer.

I believe that a brief remark in regard to the measurement tubes themselves is pertinent here. We know that pipettes for measuring serum must be calibrated for accurate "intake", i.e. the serum contained from the tip to the first gradation must actually be 0.01 ccm; when expelling the fluid, the remainder adhering to the inner walls is still included in this amount but is recovered through mixing with physiological salt solution. On the other hand, pipettes for introducing the physiological salt solution, etc. must be accurately calibrated for "output", i.e. not the fluid drawn in but the amount expelled should be exactly the desired quantity. From the exterior, serum pipettes are recognized by the fact that the gradation is numbered from the tip upward whereas the opposite is true of the output pipette. Serum drawn into an output pipette from the tip to the first gradation numbered 0.9 is not 0.1 ccm.
but about 0.15 ccm because the volume in the tip is not included in gradation. Consequently, inaccurate selection or incorrect use of the pipette can create an error of about 30% which is actually overlooked in many cases. Many variations in titer are therefore, in addition to other circumstances, perhaps due to such inaccurate pipetting.

In addition to the procedures detailed above which are probably the most frequent, the device can obviously be used otherwise with the same advantages when we are concerned with measuring fluid quantities up to about 1 ccm rapidly, frequently and accurately.

We should emphasize once more that the device makes it possible to eliminate the dangerous method of pipetting by mouth, from bacteriological and seriological laboratories, without resulting in any loss of time, inaccuracy or inconvenience.

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9) u. 10) in Heim, Lehrb. d. Bakt.
13) Hoerder, Ebenda, 1909.
20) Wright, A.E., Jena 1914.