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Recommendation for a selected method for the measurement of plasma viscosity

International Committee for Standardization in Haematology*

Plasma viscosity is an important factor influencing whole blood viscosity. It is directly correlated with the concentration of large sized molecules, especially proteins. In both acute and chronic conditions changes may occur which are reflected in the measurement of plasma viscosity. No reference method has yet been defined, but the following document is aimed at providing a suitable working method—that is, "selected method"—for the measurement of plasma viscosity to ensure a meaningful comparison of data when the same procedure is used in different laboratories throughout the world. The Harkness method (see references 4 and 6) has been selected for this as it is simple to perform and appears to be well reproducible. Viscometers using different principles and dimensions but which yield identical values are equally acceptable. Other techniques may also be acceptable provided that their results coincide with those obtained by the selected method with normal and various abnormal samples. This tentative standard will be reviewed one year after publication in accordance with the rules of the International Committee for Standardization in Haematology (ICSH). Comment is invited and correspondence should be addressed to the ICSH Executive Secretary (Dr RL Verwilghen), University Hospital, 33 Capucijnenvoer, B-3000 Leuven, Belgium.

In recommending the Harkness method the panel has taken into account the following:

1 The *low cost* and *operative simplicity* of a capillary instrument.

2 *Volume of sample.* Only 0·5 ml of plasma is required to perform the test.

3 *Duration of test.* The total time per test is less than 1 min, which includes all the manipulations and clerical work; the time allocated to the viscometry is less than half of this total.

4 *Accuracy.* The procedure is capable of distinguishing between two samples of plasma where viscosities differ by 0·03 mPa.

5 The accurate *timing* by an automatic electronic system allows a short time of flow.

6 There is no need to *clean* and *dry* the viscometer between samples.

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Standard techniques before arrival of blood specimen in the laboratory

As the final viscosity values can be affected by factors introduced during the production of the plasma sample, it is also necessary to standardise the preparatory procedures.

PREPARATION OF THE PATIENT

Plasma viscosity remains remarkably constant throughout a day free from above average stimuli. However, as circadian periodicity has been observed, it is best to confine serial tests to the same hour on repeated testing. Over-hydration can produce a small but detectable reduction in the plasma viscosity, and similarly dehydration can lead to a rise in viscosity.

CONTAINERS FOR BLOOD SPECIMENS

Anticoagulant

No anticoagulant in common use is absolutely free from an effect on the viscosity of the plasma, owing to the passage of protein-free fluid into or out of erythrocytes. The recommended anticoagulant is edetic acid (K or Na salt) at a concentration of 3·4–4·8 mmol/l blood; this appears to cause least change in the plasma viscosity.

Containers

These should be small enough to leave little space above the blood sample; flat bottomed so that they can stand on the bench with minimal surface contact between the plasma and cell columns; strong enough to allow centrifugation in the original container; and with a close fitting stopper so that blood does not

seep between the stopper and the tube wall. Either glass or plastic is acceptable.

Collection of the specimen
Blood taken from any blood vessel is acceptable, but the antecubital vein is the most convenient site. The blood should be collected without stasis, using a sterile needle and dry plastic syringe. Without delay, add the exact specified volume to the container with edetic acid anticoagulant, stopper tightly and mix by 20 (actually counted) inversions of the tube; do not shake violently as bubble formation must be avoided.

SUBSEQUENT PRELABORATORY TREATMENT OF THE SPECIMEN
The amount by which the viscosity of the plasma increases depends on the duration and frequency with which the cells come in contact with the plasma. Blood centrifuged immediately after collection and left undisturbed has a plasma viscosity virtually unchanged after 24 h, whereas blood frequently remixed will have a notably raised plasma viscosity in the same time. Hence the subsequent treatment of the sample differs according to the delay before it reaches the laboratory.

1 Where the specimen will reach the laboratory within 6 h, the blood should be sent in its original container. Any unnecessary remixing of the blood should be avoided.

2 Where the specimen will reach the laboratory only after 6 h, and for all postal specimens, treatment should be as follows. If the peripheral source has its own centrifuge, centrifuge the original blood specimen as soon as convenient; transfer the plasma to a small container for dispatch to the laboratory. Otherwise, leave the original blood sample standing upright on the bench. When erythrocyte sedimentation has occurred, transfer the plasma to a small container for dispatch. Neither the blood nor plasma needs to be kept in a refrigerator.

TREATMENT OF THE SPECIMEN WITHIN THE LABORATORY
Do not remix the contents of the specimen tubes. Centrifuge both blood specimens and plasma specimens at 1500 *g* for 5 min. This will not settle the platelets, but there is no detectable difference in viscosity after centrifugation for an additional 25 min. Tubes must be capped during centrifugation. Filtration of the plasma is not necessary.

Storage
If the plasma is to be tested on the same day it may be left undisturbed in contact with the packed erythrocyte column, at room temperature. If an overnight delay is unavoidable, pipette off the plasma to a small stoppered container. Under sterile conditions this can be left on the bench at room temperature for up to one week; remix, in order to include any condensation, before testing.

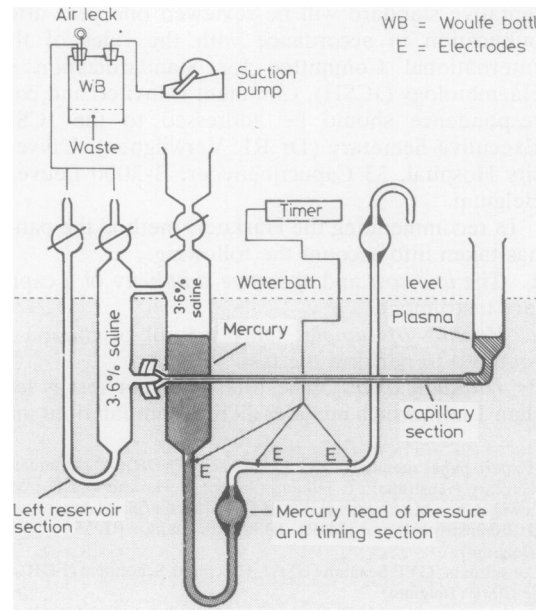
Do not freeze or store in an ordinary refrigerator as this may affect the lipid-protein complexes and thereby alter the viscosity.

Viscometry system

The viscometry system may be considered under two headings: the main viscometer and the auxiliary equipment.

VISCOMETER
There are three identifiable sections in this viscometer (Figure).

Capillary section
The capillary is made from a 200 mm length of precision glass (of uniform bore). This widens at one end to a 2 mm tube which enters the base of a specimen cup (13 mm × 70 mm) and at the other end to a 2 mm tube, by which it joins the second section by a spring loaded ball and socket joint. The specimen cup (but not the capillary itself) is siliconed to expedite fluid drainage. The straight shape of the capillary facilitates cleaning by the pas-



The Harkness viscometer

sage of a wire in the rare event of an obstinate blockage of the lumen.

Although a bore of 0·30 mm for the capillary is recommended for a reference method, a wider bore of 0·38 mm is preferred in practice for a routine method, especially when the test is performed at temperatures below 37°C.

Left reservoir

This 13 mm wide tube is closed above by a stop cock. A side arm towards the top joins this reservoir to the third section. Lower down there is the ball and socket joint for the capillary. Another tube leads from the base and is closed above by a stop cock.

Mercury pressure and timing section

This section is basically a U tube of 2 mm bore which has been modified to the shape shown in the Figure to serve three functions.

1 *Source of standard head of pressure.* The left limb is expanded into a reservoir of 17 mm diameter; it is closed above by a stop cock and funnel; the lowest part of the parallel side walls begins at a level about 1·5–2 cm above the horizontal portion of the right limb; the side arm connection to the second section is 9–10 cm above this horizontal portion; thereby, a standard head of pressure in the range of 1·5–9 cm mercury can be obtained between the meniscus of the mercury in the reservoir and the meniscus in the horizontal portion.

An electrode is inserted into the left limb.

2 *Activator of electronic timer.* Two electrodes are inserted into the horizontal portion of the right limb as part of the circuit by which the electronic timer is activated. When the mercury reaches the left electrode the timer is started, and the right electrode stops it.

3 *Measure of standard volume.* The two electrodes are inserted 6·4 cm apart to contain the standard volume (0·2 ml) between them; the passage of the mercury is accompanied by a parallel passage of test fluid through the capillary.

AUXILIARY EQUIPMENT

Support for viscometer glass parts

The glass parts are held at a distance from a rigid metal or plastic backing plate; these distant pieces consist of metal or plastic clips at the end of threaded rods, which are passed through slots in the backing plate and adjusted into position by nuts and washers, front and back. The considerable weight of the mercury section is carried by the bottom of its U tube resting on a shelf bolted to the backing plate. The outer end of the capillary is supported similarly by a shelf. In turn, the backing plate is supported either by arms resting on the upper rim of the water bath or by vertical rods rising from a base plate.

It is essential that the mounting system is free of any sources of vibration such as an electric vacuum pump, water circulator, mechanical timer, and a timer attached to the backing plate. A simple test for vibration is to examine the surface of the mercury for ripples in an appropriately directed beam of light.

Standard temperature: working temperature

The ideal standard temperature is that of the original body temperature of 37°C, and all plasma viscosity results should be reported in terms of 37°C. In practice, however, this is not the most convenient temperature for routine testing since it is more expensive to produce an accurate temperature which differs from the ambient temperature, and the routine testing will be delayed considerably by the time necessary to raise the temperature of each specimen to 37°C and to maintain it at that temperature. Practical experience has shown that as the temperature selected becomes higher the chances of introducing experimental errors increase. There is no clinicopathological advantage to be gained by interpreting the plasma viscosity measured at any particular temperature within the 20–37°C range. In working conditions the temperature dependency of the plasma viscosity is approximately identical to that of water, and plasma viscosity may be measured at any desired temperature and then corrected by the ratio of water viscosity at 27°C to water viscosity at the temperature of measurement. Between 20°C (1·0 mPa.s) and 37°C (0·6947 mPa.s) the viscosity of water increases linearly. Except for a few cases of myeloma and macroglobulinaemia where cryoproteins are present in the plasma, each degree centigrade change of temperature causes a 2·4% change in viscosity. Thus for the working temperature there are three choices.

1 Despite the difficulties noted above the preferred method is to undertake the extra effort and work at 37°C.

2 The simplest method is to measure at the ambient temperature and make the appropriate mathematical correction to 37°C to each value.

3 The most convenient method is to add a temperature controlling system at a few degrees above the ambient temperature and modify the mercury head of pressure as the temperature correction factor (see below). A constant temperature water bath is more convenient, more accurate, and less expensive than a constant air temperature room or cabinet. A simple and economical arrangement consists of an all glass tank or a glass sided fish tank, with the water

heated and circulated by a combined unit; a reasonably priced thermostat will maintain the temperature to within ±0·05°C or better. For each degree centigrade below 37°C a correction of +2·4% of the measured viscosity must be added.

Adjustable pressure source and waste receiver
A small electric suction pump is attached by plastic or firm rubber tubing to a Woulfe bottle, where the pressure is adjustable by a spring loaded air leak. The Woulfe bottle acts also as a receptacle for the waste plasma specimens after testing.

Timing system
A timer should be accurate to 0·01 s or to 0·0001 of the time of flow. A variety of systems can be used: the simple, cheap, and easily constructed unit described by Manson[7] is recommended.

Mercury
Mercury of the highest purity is essential for a freely flowing timing system. It is recommended that two volumes be kept in rotation—one in the viscometer, while the other is being cleaned, using for this purpose any standard method, as described in chemistry texts.

Priming fluid and viscosity standard
1 Priming fluid. 36 g/l NaCl ("saline") has a specific gravity close to the average plasma (1,0255) and thus the hydrostatic balance within the viscometer is maintained.
2 Viscosity standard. Although a solution with a viscosity about 50% higher would be preferable, 36 g/l NaCl saline has proved to be a convenient standard (0·732 mPa.s at 37°C; 0·943 mPa.s at 25°C).

Empirically it has been found that this saline appears to prevent the deposit of protein from the plasma on to the wall of the specimen cup or capillary—hence these must be left filled with fresh saline when not in use.

The saline solution is prepared from NaCl (chemically pure grade) dissolved in freshly distilled water; it is filtered through a hard filter paper and kept dust free in plastic squeeze bottles.

Transfer pipettes
Glass Pasteur pipettes are the most common source of trouble in viscosity measurements because of the presence of traces of packing material and glass spicules, which block off the capillaries. A batch of plastic Pasteur type pipettes, graduated at 0·5 ml, should therefore be reserved for viscosity work. After each use they should be rinsed clean with water and dried. A stock should be kept in a wide mouthed jar immersed in the water bath so that the standard temperature of the specimen will be maintained.

Specimen rack
A 10-holed rack should be submerged at the surface of the water bath to hold the plasma specimens for a minimum of 10 min while the plasma equilibrates with the temperature of its surrounding water.

Technique of viscosity measurement

GENERAL
The principle of the recommended viscometer (Figure) is as follows. On opening the stop cock at the top of the left reservoir the negative pressure in the Woulfe bottle will tend to pull out the priming fluid and raise the mercury in its reservoir; the air leak is adjusted to bring the right mercury meniscus just to the left of the left timing electrode in the horizontal portion. On closing the stop cock the mercury head of pressure will attempt to regain equilibrium and thus exerts a negative pressure to pull the contents of the specimen cup through the capillary; the difference between the levels of the mercury in the wider reservoir and the horizontal timing portion is the standard pressure. The standard volume is the volume between the two timing electrodes since in the "time of flow" an identical volume of test fluid will have passed along the capillary.

When the timing is completed, the fluid remaining in the specimen cup is immediately pipetted to waste so that as the mercury continues to rise in the right limb towards equilibrium, the liquid in the 2 mm tube below the specimen cup will also be pulled into the capillary. By the time the operator is ready to introduce the next specimen the residue of the previous specimen will have drained down from the siliconed cup wall and will be lying next to the capillary entrance. When the following specimen (0·5 ml) is pipetted gently into the base of the cup an air bubble is trapped between it and the capillary. Then, as the stop cock above the left reservoir is opened, the last trace of the previous specimen passes into the capillary, followed by the air bubble, and followed by the meniscus of the new specimen, which effectively scours the last traces of the previous specimen off the capillary wall. The moment when the stop cock should be closed is determined by experience: by the time the mercury meniscus reaches the left electrode the whole air bubble should have entered the left reservoir and risen to lie below or pass through the stop cock. The presence of an air bubble in the hydrostatic system does not produce an error since the pressure is virtually constant throughout the test.

Since the diameter of the specimen cup is rela-

tively large and as there is no air-fluid interphase in the capillary, no allowance need be made for the effects of surface tension.

CALCULATIONS

The viscosity of the test fluid is calculated from a comparison of its time of flow with that of a fluid of known viscosity, under the same standard conditions.

$$\text{Viscosity of test fluid} = \frac{\text{Viscosity of standard} \times \text{time of flow of test}}{\text{Time of flow of standard}}$$

It is possible to avoid calculations by adjustment of the mercury pressure.

Adjustment to exclude viscosity factor

This is based on producing a time of flow in seconds which is exactly 10 times the viscosity in mPa.s; viscosity is obtained by moving the decimal point one place to left. Viscosity of 36 g/l NaCl is 0·732 mPa.s at 37°C, and 0·943 mPa.s at 25°C.

Begin with a head of pressure of about 6 cm mercury. Adjust the volume of mercury by abstracting through a fine plastic tube introduced through the lumen of the upper stop cock or by adding from the funnel until the time of flow (mean of 10 tests) is within 0·02 s of expected times.

Adjustment for temperature correction

Similarly, if the time of flow for 36 g/l NaCl at 25°C is reduced to 7·32 s by increasing the mercury pressure then the timer will present the viscosity in terms of 37°C values, and the actual 25°C values will have to be calculated.

DETAILED INSTRUCTIONS FOR THE MEASUREMENT OF VISCOSITY

It is assumed that the viscometer and the auxiliary equipment have been assembled properly and calibrated at the selected standard working temperature.

Preliminary procedure

1 Turn on the electric supply to the auxiliary equipment.
2 Place nine plasmas and one 36 g/l NaCl (saline) sample in the rack in the water bath.
3 Check that there is an adequate supply of transfer pipettes in the container in the water bath.
4 Check that the negative pressure is properly controlled.
5 Check that the correct working temperature has been attained and maintained.
6 Check that the capillary has no visible dirt or obstruction.
7 Transfer 0·5 ml of standard saline to the specimen cup and measure the time of flow (see below). This should be within ±0·1 s of the mean standard time.
8 If the time difference is small, adjust by small side wedges which tilt the whole instrument and thus makes small changes in the head of pressure.
9 If the time difference is large, remove the capillary for mechanical or chemical cleaning, or both.

Test procedure

1 Open tap at top of left reservoir until all the residual saline has entered the capillary. Close tap.
2 Transfer 0·5 ml of the first plasma sample to the base of the specimen cup, trapping an air bubble in the tube below.
3 Open the top tap long enough for the mercury meniscus to reach equilibrium point and for the air bubble and a little plasma to pass through the capillary.
4 Close tap; reset timer to zero.
5 When timer has stopped, pipette residual plasma to waste.
6 Record the time of flow and make any appropriate calculations.
7 Return to stage 2 with the next specimen.
8 Continue to test the batch for as long as the results are normal or intermittently moderately abnormal.
9 If a very high result is found (that is, 1·9 mPa.s at 37°C) or if a series of three moderately abnormal values occur, inspect the capillary for blockage and make a repeat test with standard saline. Should the saline result be too high, remove the capillary for cleaning.
10 On the completion of the testing, fill the funnel above the mercury reservoir with saline and flush the left reservoir thoroughly. Fill the specimen cup with saline and leave it capped to keep out the dust.

Reference values

The figures given below were established for a British population. For other population groups appropriate reference values should be prepared.

The newborn have plasma viscosities in the range of 0·98–1·25 mPa.s at 37°C. By the third year these values have risen to adult levels (1·16–1·33 mPa.s at 37°C; 1·50–1·72 mPa.s at 25°C), where they remain until late middle age, when the slowly rising fibrinogen makes them somewhat higher. The ranges are the same for both sexes.

SIGNIFICANT CHANGE

A persistent change of 0·03–0·05 mPa.s is indicative of a significant change in the metabolism.

SIGNIFICANCE OF VALUES OUTSIDE REFERENCE VALUES

Organic diseases (for example, infection, trauma, malignancy) which produce a systemic reaction cause a rise in the plasma viscosity and the extent of the rise is related to the severity of the condition.

Acute diseases, such as pneumonia and cardiac infarction, have values within the range of 1·35 to 1·95 mPa.s at 37°C. Chronic diseases, such as connective tissue disorders and fibrotic pulmonary tuberculosis, have values which are generally restricted to the lower levels of 1·35 to 1·55 mPa.s at 37°C.

Paraproteinaemias, such as myeloma and macroglobulinaemia, have characteristically high values of above 2·0 mPa.s when the protein changes in the blood are fully developed.

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Clinical

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