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Laboratory techniques

Guidelines on selection of laboratory tests for monitoring the acute phase response

INTERNATIONAL COMMITTEE FOR STANDARDIZATION IN HAEMATOLOGY (EXPERT PANEL ON BLOOD RHEOLOGY)

SUMMARY These guidelines refer to laboratory tests for monitoring changes in acute phase proteins in patients with an inflammatory response to tissue damage. Quantitative measurements of acute phase proteins are a valuable indicator of the presence, extent, and response of inflammation to treatment. When short term (less than 24 hours) changes in the inflammatory response are expected, quantitative assay of C reactive protein is the test of choice. The hyperproteinaemia that develops in response to a longer term (more than 24 hours) inflammatory response is complex and may vary from one disease to another. A test that is sensitive to the combined effect of several plasma proteins is therefore indicated, and appropriate tests include the erythrocyte sedimentation rate and plasma viscosity—the latter having several advantages. Tests for monitoring short term and long term changes in acute phase proteins are complementary and should be used for different clinical purposes; no one test is ideal for all clinical situations. A quality control programme is an essential component of laboratory tests for monitoring the acute phase response.

The inflammatory response to tissue injury includes a change in the plasma concentration of several proteins that originate in the liver and which are known as acute phase proteins. This phenomenon is part of a wider response (acute phase response) which includes fever, a leucocytosis, and increased immune reactivity. The acute phase response is mediated by cytokines—for example, interleukin-1 released from macrophages and possibly other cells. The definition of the "acute phase" has been imprecise; it should be used to reflect the intensity of the inflammatory response rather than its duration as the changes described occur in both acute and chronic inflammation. Thus a patient with chronic disease may show periodic or continuous evidence of the "acute phase" response depending on the activity of the inflammatory response.

The plasma concentration of an acute phase protein may increase—for example, C reactive protein and fibrinogen—or decrease—for example, prealbumin and albumin. Laboratory measurement of acute phase proteins is a valuable indicator of the presence, extent, and response of inflammation to treatment. Although specific laboratory assays are now available to measure individual acute phase proteins, the erythrocyte sedimentation rate (ESR) is still widely used as a non-specific test to measure the combined effect of some of these proteins on erythrocyte aggregation. Measurement of plasma viscosity, which also reflects the cumulative rheological effect of some of these proteins, is used as an alternative to the ESR. Both tests have the advantage that they are technically simple to do.

In previous published reports the International Committee for Standardization in Haematology (ICSH) has recommended methods for the measurement of the ESR[1] and plasma viscosity.[2] These reports are reviewed in the present guidelines.

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Erythrocyte sedimentation rate

The ICSH recommendation published in 1977 on measurement of the ESR was for a selected ("routine") method based on that of Westergren.[1] Because of the complexity of factors that determine the ESR and lack of understanding of their interaction, it was not, and still is not, possible to recommend a definitive reference method. The recommendations of this 1977 report remain valid.

BASIS OF THE ESR

The essential principle of the ESR test consists of

establishing a vertical column of anticoagulated blood and allowing the erythrocytes to settle under gravity. After a standard waiting period (one hour) the distance between the surface meniscus and the upper limit of the erythrocyte column is measured. This is the ESR.

The fact that erythrocytes settle at all under these circumstances is due to a difference in density between cells and suspending plasma, but the two factors that are particularly important in influencing the ESR are the degree of red cell aggregation and the packed cell volume (PCV).

The formation of erythrocyte aggregates (rouleaux or clumps) has a major effect on the ESR; a quantitative increase in aggregate formation or the formation of abnormal types of aggregates causes an increase in ESR. The adhesive forces between erythrocytes that cause aggregation result from the presence in blood of large plasma proteins such as fibrinogen, α_2-macroglobulin, and some immunoglobulins. The ESR therefore provides a useful, non-specific means of monitoring a variety of clinical conditions in which these plasma protein concentrations are disturbed.

Changes in PCV have a strong influence on the ESR. Decreasing the volume which erythrocytes occupy in a blood sample decreases the velocity of the upflowing plasma which the sedimenting erythrocytes displace. Thus the erythrocyte aggregates fall faster. They also fall further before bottoming-out occurs. For both these reasons a decrease in PCV will raise the ESR, independently of the changes in acute phase reactants. Changes in erythrocyte size, shape, deformability and density also influence the rate of sedimentation.

Yet another factor influencing the ESR is the viscosity of plasma. Changes in viscosity, like changes in PCV, affect the ESR in at least two ways. An increase in plasma viscosity retards the erythrocytes and thus other things being equal, should lower the ESR. Such increases in viscosity, however, usually occur because of a rise in plasma concentration of asymmetrical protein molecules which strongly accelerate the ESR. The latter effect is typically much more pronounced than the former and it is for this reason that ESR and plasma viscosity results parallel each other closely.

MODIFICATIONS OF THE METHOD

Several variations of the ICSH selected method have been developed which are primarily intended to increase convenience and safety in performing the test.

Biohazard considerations

Every effort should be made to minimise the potential hazard to laboratory staff of manual handling of contamination of laboratory apparatus, and of the presence of open blood containers in the laboratory. Use of disposable sedimentation tubes and the development of ESR methods that reduce handling of blood by using a closed system are to be encouraged. Adequate trials must be performed, however, to prove that the results obtained are comparable with those of the ICSH selected method.

The ESR should not routinely be performed on blood samples from patients who show a positive test for hepatitis virus or human immunodeficiency virus (HIV), or who are infected with any other biohazardous pathogen. As blood samples may be submitted for ESR analysis before it is established that they contain such a pathogen, all ESR samples should be handled as potentially infectious.

Disposable sedimentation tubes

Disposable plastic or glass sedimentation tubes are now widely available. Some of the plastics used (such as polypropylene, polycarbonate) are suitable substitutes for glass but not all plastics are equally suitable. Some have adhesive properties towards blood cells and some release plasticisers that affect the blood and thus the ESR. Whenever disposable tubes are used they should be supplied clean and dry and ready to use; it is necessary to show that neither the tube material nor the manufacturer's cleaning process affects the ESR and that the results are comparable with those of the ICSH selected method.[1] When disposable glass tubes are used, special care is required over their disposal owing to the biohazard risk of broken glass.

Vacuum extraction systems

These systems minimise biohazard risks but the dimensions of vacuum extraction tubes, which are subsequently placed vertically to function as the ESR tube, may not accord with ICSH recommendations. As with manual methods, there is a risk of variation in the ratio of blood sample to anticoagulant diluent. While further development of this type of approach is to be encouraged, it must be shown that the results obtained are comparable with those of the ICSH selected method over an adequate range of measurements.

Micromethods

Micromethods, based on tubes that are short or of narrow bore, or both, have been proposed, mainly for tests on children. It is important to check that the results, particularly at high ESR values, are comparable with those of the ICSH selected method.

COMPARABILITY BETWEEN METHODS AND QUALITY CONTROL

It is essential to ensure comparability between

methods and to protect against the most common errors in the performance of the ESR. The latter include (i) dilutional errors (diluent improperly prepared, inaccurately measured, or inadequately mixed with the sample); (ii) faulty equipment (use of a sedimentation tube that is too narrow, dirty, or made from materials that interact with the sample); and (iii) inadequate mixing of the sample before filling the sedimentation tube or slow drawing of the sample into the tube. The procedures described below, which are intended to guard against these errors, are based on a method proposed by the National Committee for Clinical Laboratory Standards.[3]

Comparability

Intermethod comparability is most conveniently achieved by comparing results obtained by the routine laboratory method with those obtained on *undiluted* blood samples of PCV 0·33 ± 0·03 under standardised conditions in a Westergren tube that meets ICSH specifications.[1] These blood samples should be representative of the ESR range of 15–105 mm/one hour. At this PCV, undiluted whole blood samples have been shown to sediment reproducibly in glass tubes that meet ICSH recommendations[4] and to show a linear response to an increasing concentration of asymmetrical macromolecules.[5] The commonly used routine methods for measuring the ESR are also linear in at least part of the clinical range; within this range sedimentation of the undiluted whole blood sample in an ICSH tube will be linearly related to that of the routine ESR method.

The comparability procedure for the Westergren ESR method requires a set of 10 blood samples with a PCV of, or adjusted to, 0·33 ± 0·03 and ESR values that represent the range 15–105 mm/one hour. The ESR is measured on this blood by the routine Westergren method and also on an aliquot of the same specimen, *undiluted*, in a Westergren sedimentation tube specified by ICSH under strictly controlled conditions.[1] The results for the routine method are related to the undiluted ESR as follows:

routine Westergren ESR* =
(undiluted Westergren ESR * × 0·86) − 12
* expressed in mm/one hour

If the routine method is performing satisfactorily the difference between it and the undiluted Westergren ESR (corrected for lack of dilution using the above equation) should not exceed 12 mm/one hour at any point in the range 15–105 mm/one hour. A smaller difference would be expected at the lower end of this range. All samples in which the undiluted ESR is less than the routine value should be remixed with the remaining portion of the original specimen and set up once more. If the routine Westergren ESR result is still greater than the undiluted Westergren ESR, the dilution and mixing steps of the routine method should be meticulously re-examined.

Some modified ESR methods give values for sedimentation at one hour that differ from the ICSH selected (Westergren) method, and the manufacturer may provide a conversion factor. There should be published evidence of the validity of this conversion over an ESR range of 0–100 mm/one hour. The converted values should be used in the above comparability procedure.

Laboratories that use the Wintrobe ESR method or the zeta sedimentation ratio (ZSR) method may also use the above procedure. As the Wintrobe ESR at PCV values of 0·33 ± 0·03 no longer shows a linear response beyond values of 40 mm because of the shortened tube (100 mm), a more complex equation is required to fit this curvilinear response:

routine Wintrobe ESR* = −0·004 ×
(undiluted Westergren ESR*)² +
0·945 (undiluted Westergren ESR*)
− 25·8 (PCV†) + 5·56
*expressed in mm/one hour
†expressed in SI units.

The Wintrobe ESR is satisfactory if the difference from the Westergren measurement on undiluted blood, adjusted according to the above equation, is not greater than 7 mm.

The ZSR incorporates a correction for PCV; the undiluted ESR does not. Thus for comparability purposes, it is necessary to nullify the correction for PCV and return the ZSR result to that actually measured, the Zetacrit. The equation for comparability of ZSR results is:

Zetacrit =
(undiluted Westergren ESR* × −0·31) + 80
*expressed in mm/one hour

The ZSR method is satisfactory if the Zetacrit difference from the undiluted Westergren ESR, adjusted according to the above equation, is not greater than 6 units.

Whenever there is any planned change in the routine method—for example, in its location, in the type or source of ESR tubes, or in the supplier of blood sample tubes—a comparability check should be performed as above on at least 10 blood samples of a wide range of ESR values (for example, 15–105 mm/one hour).

Once a satisfactory ESR method has been established, a quality control procedure is required to check against errors in performance of the ESR.

Quality control

Quality control should be performed by testing one blood sample on each day that routine ESR tests are performed. Select a blood sample with a PCV of between 0·30 and 0·36 and perform the ESR by the

routine method and by the undiluted Westergren ESR method. Apply the appropriate formula to obtain the corrected ESR for the undiluted sample. The test is under satisfactory control if this result does not differ from that obtained by the routine method by more than 12 mm (Westergren), 7 mm (Wintrobe), or 6 units (Zetacrit). As already noted, a smaller difference would be expected for ESR values at the lower end of the range 15–105 mm/one hour (Westergren).

FACTORS AFFECTING THE ESR

Several factors, other than plasma proteins, can influence the ESR and cause problems in interpreting abnormal results.

Packed cell volume

Sensitivity of the ESR to PCV is a major problem. This is largely due to ill defined effects on the ESR of variation in erythrocyte size, shape, deformability, and density. For certain research studies it may be useful to adjust anaemic blood samples to a standard PCV of 0·35 before testing[6] but this is too tedious for routine diagnostic laboratories and detracts from the technical simplicity of the ESR.

The ESR is not, therefore, the test of choice when studying conditions that are associated with moderate or severe anaemia, or when there is a wide fluctuation in PCV.

Viscosity of the plasma

As the erythrocytes in an ESR tube must sediment through the plasma suspending phase the viscosity of the plasma will influence the ESR. It is still not clear, however, how to correct for variation in plasma viscosity because some proteins that affect plasma viscosity may not influence erythrocyte aggregation. For this reason, and to maintain technical simplicity, the effects of plasma viscosity are ignored.

The ESR is, therefore, not the test of choice when studying patients in whom plasma viscosity may be very high—for example, paraproteinaemias—or varies widely. Under these conditions direct measurement of plasma viscosity gives more useful information.

Temperature

An increase in temperature usually increases the ESR, but not always; the presence of cold agglutinins active at room temperature can impair sedimentation. Ideally, the ESR should be performed in an environment that is controlled for temperature, but in the interests of simplicity and convenience the test can be performed at room temperature (defined as 18–25°C). If even this temperature range proves impossible, a reference range should be established for the local range of ambient temperature and the temperature stated with each ESR result.

Other factors

The reference range for the ESR is different for men and women, which is only partly explained by the sex difference in PCV. Hence reference ranges should be established for each sex.

Age also has an effect on the ESR. There is a small but steady increase between the ages of 20 and 50, thereafter the increase is more pronounced. It is recommended, therefore, that reference ranges be established for different age groups. The ESR of the newborn is usually low in comparison with the adult because of the lower plasma concentration of fibrinogen and other proteins at birth.

REFERENCE VALUES

Reference ranges for the ESR are not given in these guidelines as they are influenced by local conditions as indicated above. A range that includes 95% of values from a representative local healthy population should be established.

DELAY AFTER VENEPUNCTURE

As previously recommended,[1] the ESR test should begin within two hours of venepuncture for blood samples maintained at room temperature.

Newer tests of erythrocyte aggregation

New methods for quantifying the effect of acute phase proteins on erythrocyte aggregation are potential alternatives to the ESR.

BACKGROUND

In static normal adult blood erythrocytes spontaneously adhere together in aggregates to form rouleaux. The adhesive forces responsible are generated by the large plasma proteins, of which fibrinogen is the most effective but others (α_2-macroglobulin and some immunoglobulins) also contribute. Erythrocyte aggregates that are morphologically different from rouleaux may also form. Aggregate formation is a non-specific index of the progress of clinical conditions associated with an acute phase increase in plasma proteins. Aggregates have an important influence on blood rheology, increasing blood viscosity at low shear rates and being largely responsible for the viscoelastic properties of blood. Aggregation of erythrocytes is thought to endow blood with a yield stress which may influence microcirculatory flow.

Aggregate formation occurs only in static or slow moving blood because the adhesive forces are generally small; when there is rapid flow, high shear stresses swamp the adhesive cellular interactions and break up aggregates. The extent of aggregate formation depends on the nature and concentration of

the aggregating proteins present, the plasma viscosity, erythrocyte deformability, and erythrocyte surface charge density. Techniques that have been developed for estimating aggregate formation measure different aspects of the process and are therefore affected differently by the above determinants.

DIRECT MICROSCOPIC OBSERVATION

The mean number of cells for each aggregate is determined microscopically. Erythrocytes from one blood sample are suspended at a PCV of 0·01 in two suspensions. One is the control, in which the suspending phase is a non-aggregating physiological buffer containing 0·5% v/v human serum albumin. The other is the test aggregating phase, which may be plasma. Drops from each of these suspensions are added to the wells of a Neubauer haemocytometer slide. After about three minutes equilibrium aggregation is usually achieved and photomicrographs are taken. Counts are then made from the micrographs of the number of cellular units—a cellular unit is defined as a single discrete cell or a single aggregate. Hence the mean number of cells for each rouleau can be calculated from:

$$\frac{\text{Number of cellular units in the control sample}}{\text{Number of cellular units in the test sample}}$$

and is referred to as the microscopic aggregation index.

This is a tedious and technically demanding technique of limited use to the routine clinical laboratory. It is important for two reasons. Firstly, because aggregation is measured at equilibrium and is therefore not affected by the viscosity of the suspending phase. Secondly, because the end point is based on direct observation of aggregates. The technique does not, however, reflect the kinetic or dynamic aspects of cell aggregation and may be insensitive at abnormally high concentrations of fibrinogen. As the gap between the haemocytometer slide and cover slip is only about 100 μm, the method may also not accurately reflect large aggregates that have a three-dimensional configuration.

VISCOMETRY AT LOW SHEAR RATE

The major factor responsible for the rapid increase in apparent viscosity of blood as shear rate falls below about 5 s^{-1} is the formation of erythrocyte aggregates. Guidelines on the methodology for measurement of blood viscosity have been prepared by ICSH,[7] and it is recommended that measurements at low shear should be made as near as possible to 1 s^{-1}.

Measurements at low shear depend critically on PCV and plasma viscosity. As a first approximation, however, a useful derived index of aggregation (viscometric aggregation index) can be obtained from the ratio of the low shear (1 s^{-1}) to high shear (200 s^{-1}) viscosity measurements, which gives good correction for variation in plasma viscosity and some correction for PCV. There is, however, little clinical experience of this index.

DETERMINATION OF VISCOELASTICITY OF BLOOD

Blood possesses viscoelastic properties and analysis of the stress: strain relations of the response to oscillatory deformation allows the viscous and elastic terms to be obtained. The elastic component can be a sensitive index of the formation of erythrocyte aggregates. A standard Couette viscometer to which an oscillating accessory is attached may be used to impose oscillatory deformation and determine the resulting stress. Oscillatory deformation may also be applied to a pressure chamber attached to a capillary system to strain the sample; the generated pressures are then measured. As with other viscometric techniques, these methods are very sensitive to PCV. They are also prone to the effects of cell settling (especially the capillary method) which can lead to artefacts in strongly aggregating samples. Clinical experience of these methods is limited.

OPTICAL MEASUREMENT OF ERYTHROCYTE AGGREGATION

A sample of blood is placed in the gap of a transparent cone-and-plate shearing system and first subjected to high shear to disrupt erythrocyte aggregates. Shearing is then stopped abruptly, or reduced to a very low level, to allow reaggregation to take place. The change in transmission, or in the extent of back scattering, of a beam of light passing through the sample reflects the rate and extent of erythrocyte aggregation. This technique is, however, sensitive to PCV and is also influenced by plasma viscosity.

ZETA SEDIMENTATION RATIO

In this test the blood samples are introduced into capillary tubes which are then sealed at the lower end and spun in a centrifuge (Zetafuge). The spinning axis is essentially vertical, the upper end of each tube being canted inwards slightly to prevent centrifugal force from emptying the tube of its contents. Under the 7–8 *g* produced by the centrifuge, the cells travel to the outer wall of the tube and aggregate. After 45 seconds, the tubes are rotated through 180° and the process repeated. Four spinning cycles take place over three minutes. During each cycle the cells disperse and then aggregate and continuously fall under the downward 1 *g*. Thus they follow a zig-zag course down the tubes.

At the end of the spinning period the apparent PCV is measured. This is referred to as the Zetacrit. The index of aggregation, the ZSR is defined as:

$$\text{ZSR (\%)} = \frac{\text{PCV*} \times 10^4}{\text{Zetacrit}}$$

*expressed in SI units

The PCV may be determined in the same tube as the Zetacrit by subsequent high speed centrifugation (> 10 000 *g* for five minutes) or may be determined by any suitable routine method on another aliquot of the same blood sample.

CONCLUSION

New methods that give precise quantitative measurements of the kinetics, extent, or structure of erythrocyte aggregates are potential replacements for the ESR. Dependency on PCV is a major limitation as it is with the ESR. Further development of instruments for measuring the ZSR would seem to be of particular value as the test is insensitive to PCV over the range 0·25–0·47, and clinical samples outside this range can be readily brought within it by arbitrary removal of erythrocytes or plasma, without affecting the results. Published studies, using a previously available commercial instrument, have indicated the clinical value of ZSR measurements.

Plasma viscosity

The earlier ICSH recommendation[2] of a selected method for measuring plasma viscosity referred exclusively to the Harkness capillary viscometer. The present report applies to the wider range of viscometers that are now available.

PREPARATION OF PLASMA SAMPLES

ICSH guidelines on the collection of blood samples for rheological study[7] also apply to the measurement of plasma viscosity. The anticoagulant of choice is dipotassium ethylenediamine tetra-acetic acid at a concentration of 2·4–4·8 mmol/l blood as it does not cause precipitation of proteins on storage. The blood and anticoagulant should be carefully mixed as formation of a fibrin clot will invalidate the test. Anticoagulated specimens should be centrifuged as soon as possible (never later than six hours after venepuncture) and the separated plasma removed. Once separated, its viscosity may remain stable for several days if sterile and kept at an ambient temperature of 15–20°C. Viscosity measurements are not reliable if the plasma is refrigerated as cryoproteins may be present. Stored plasma and specimens received by mail can also be unreliable because of the risks of bacterial contamination, protein degradation, and fibrinolysis.

BASIS OF PLASMA VISCOSITY

Plasma viscosity primarily depends on the concentration of plasma proteins of large molecular size, especially those with pronounced axial asymmetry—that is, fibrinogen and some immunoglobulins. Because of the lack of fibrinogen, serum viscosity is lower than plasma viscosity, but the same methods are used for measuring plasma viscosity and serum viscosity.

Plasma and serum are Newtonian fluids—that is, their viscosity values do not vary with the rate of shear—except in very rare instances when abnormal plasma proteins, such as paraproteins and cryoglobulins, form shear-dependent reversible aggregates. This Newtonian behaviour simplifies the determination of plasma viscosity in that measurements need not be carried out at different shear rates.

ROTATIONAL VISCOMETERS

When using rotational viscometers at a constant shear rate, or a constant shear stress, viscosity is calculated as the ratio of shear stress to shear rate:

$$\text{Plasma viscosity } (\eta_p) = \frac{\text{shear stress } (\tau)}{\text{shear rate } (\gamma)}$$

$$= K \times \frac{\text{torque}}{\text{rotational speed}}$$

where K is a conversion factor dependent on the geometry of the viscometer system; it can be determined using Newtonian standard fluids with viscosity values that cover the range of plasma viscosity to be measured. It is recommended that water (viscosity 0·692 mPa·s at 37°C) be used as the low viscosity standard and a freshly prepared 28% w/v sucrose solution (viscosity 1·972 mPa·s at 37°C) be used as the high viscosity standard.

When measuring plasma viscosity using a rotational viscometer, a major concern is the formation of a surface film at the air-liquid interface which can lead to an erroneously high reading, especially at low shear rates. A guard-ring is often used to prevent this shear-dependent increase in plasma viscosity but alternative methods may be suitable.

Another necessary precaution is to avoid Taylor vortices and the attendant false increase in viscosity. These vortices develop when using high shear rates to measure fluids of low viscosity. By determining the viscosity of water or an appropriate sucrose solution[8] over the high shear rate range of the instrument, the upper limit of shear rate to be used can be determined.

Because the viscosity of plasma is lower than that of blood, a lower stress value is generated at any given shear rate. It is, therefore, generally advisable to make plasma viscosity measurements at high shear rates, provided that Taylor vortices are avoided. In some rotational viscometers procedures aimed at protecting against vibration and draughts, as described for blood viscosity measurements,[7] also apply.

CAPILLARY VISCOMETERS

In capillary viscometers plasma viscosity is determined

from the relation between pressure (P) and flow rate (Q):

$$\text{Plasma viscosity } (\eta_p) = K' \times \frac{P}{Q}$$

where the geometric constant K′ is determined from the P-Q data obtained using Newtonian standard fluids of known viscosity in the same viscometer.

For plasma, the P-Q relation can be determined using a capillary tube through which the plasma is perfused at a constant flow rate or at a constant pressure head. Because plasma viscosity is generally independent of shear, it can also be measured under conditions in which P or Q change. An example of this approach is the U-tube system in which the plasma is allowed to flow by gravity from one arm of the U-tube to the other as a consequence of the hydrostatic pressure difference created by filling the two arms with the sample to different heights. The most common method of determining plasma viscosity in such a system is to measure the time (t_p) required for the sample to traverse between two marked heights; the time (t_w) required for water, or another Newtonian standard fluid of known viscosity, to traverse between the same marks is also measured. Plasma viscosity is calculated as:

$$\text{Plasma viscosity} = \text{water viscosity} \times \frac{t_p \times d_p}{t_w \times d_w}$$

where d_w and d_p are the density values of water and plasma, respectively. The density of water is 0·993 at 37°C and 0·997 at 25°C. The density of plasma is a function of plasma protein concentration, but the use of values of 1·023 for 37°C and 1·027 for 25°C will introduce errors of less than 1%.

To avoid the effects of surface film formation it is recommended that the difference in height between the fluid columns in the two arms of the U-tube be no less than 10 cm throughout the test.

In all capillary viscometers the ratio of the capillary length to diameter should be not less than 40 to minimise the influence of the capillary entrance region on the measurement. Care should be taken to eliminate air bubbles in the capillary.

ROLLING BALL VISCOMETERS

In addition to rotational and capillary viscometers, plasma viscosity measurements can be made using a rolling ball viscometer. In this instrument a metal ball is placed in a cylindrical tube and the tube then filled with plasma. To assure rolling rather than falling of the ball, the tube is tilted slightly (10–15°) from the vertical. After temperature equilibration of the test sample the metal ball is raised from the bottom of the tube, then released, and allowed to roll downward; timing of the motion of the ball over a fixed distance is made using electronic sensors. Calculation of plasma viscosity from this time measurement requires prior calibration of the viscometer with fluids of known viscosity, following which it is possible to obtain a calibration constant for the ball-tube system. Multiplication of the measured time for plasma by this calibration constant and the difference in density between the metal ball and plasma yields the value for plasma viscosity.

As the metal ball is always located within the liquid being tested, rolling ball viscometers are not subject to artefacts due to surface film formation at the air-liquid interface. They are, however, sensitive to the presence of air or gas bubbles on the inside walls of the tube which can retard the rolling motion of the ball.

BIOHAZARD CONSIDERATIONS

In all viscometers the part of the instrument in contact with the plasma sample should be readily accessible for cleaning after each test, or should be disposable. After cleaning, the surfaces should tolerate sterilisation with a disinfectant that is effective against hepatitis virus and HIV. As few viscometers have been designed primarily for bioassays on blood specimens this has limited their use in hospital diagnostic laboratories. Design of a fully automated plasma viscometer, including the centrifugation step, for safe handling of human plasma would be a major advance.

CONTROL OF TEMPERATURE

Plasma viscosity varies inversely with temperature; viscometers must therefore be capable of maintaining a constant temperature (± 0·5°C or better) of the plasma sample in the instrument. When cryoproteins are absent, the viscosity of plasma and water change in parallel with a change in temperature so that plasma viscosity can be measured at any temperature between 20 and 37°C, provided it is expressed relative to the viscosity of water. In keeping with ICSH guidelines for measurement of blood viscosity,[7] it is recommended that measurements be made at 25°C or 37°C. In all cases the temperature at which the measurement was made should be clearly stated; the ability to monitor the working temperature of the test sample is a desirable feature of an instrument. In samples containing cryoproteins measurements made at several temperatures will provide valuable information.

EXPRESSION OF RESULTS

The absolute unit (International System of Units or SI) of plasma viscosity is milliPascal seconds (mPa.s) which is equal to centipoise in the former centimetre, gram, second (CGS) system. While the use of this unit has the advantage that results can be compared with those for whole blood viscosity, expression of the viscosity of plasma relative to water has the merit that this ratio is usually independent of temperature. It

may, therefore, be useful to report both the absolute value (along with temperature of measurement) and the value relative to water.

The normal plasma viscosity averages 1·24 mPa.s at 37°C and 1·60 mPa.s at 25°C. Each laboratory should establish its own reference values.

EVALUATION OF VISCOMETERS

The following procedures for the evaluation of viscometers have been adapted from the ICSH protocol for evaluating automated blood cell counters[9]; analysis of results should be carried out as described in that document.

Precision

Measure 10 aliquots of one normal plasma specimen consecutively to obtain the intrabatch coefficient of variation (CV) of the method.

Obtain a sample of plasma of high viscosity and prepare a series of five to six dilutions by adding appropriately increasing volumes of plasma of low viscosity (such as a plasma protein fraction) to obtain a range of viscosities. Measure these in duplicate in several successive test batches. Calculate the interbatch CV at different levels of viscosity.

Carry-over

Determine this by testing high and low samples in sequence. The low sample may be a plasma protein fraction.

Instrument performance

A record should be kept of all breakdowns and technical difficulties encountered during the evaluation.

Assessment of efficiency

To assess throughput determine the number of samples (including standards and controls) that can be processed for each unit of time.

Quality control

At least one normal and one abnormal standard should be included in each batch of test samples and the results plotted on a quality control chart.

Reference values

A reference range that includes 95% of values from a representative local healthy population, including the elderly, should be established. Plasma viscosity is lower in neonates than in adults because of the lower concentration of plasma proteins, particularly fibrinogen, and reaches adult values by about 3 years.[2] Viscosity increases slightly in the elderly as the plasma fibrinogen increases. There is no difference in plasma viscosity between men and women.

Assay of individual acute phase proteins

Whereas non-specific tests such as the ESR and plasma viscosity are sensitive to the cumulative effect of several plasma proteins, immunoassays for individual acute phase proteins are now available. To avoid the need to assay multiple proteins it is important to identify those acute phase proteins that are the most sensitive and specific for inflammation. Several criteria must be considered when selecting an acute phase protein for diagnosing or monitoring inflammation.

ONSET OF INFLAMMATION

After tissue injury the time taken for a detectable increase in plasma protein concentration to occur varies with different proteins. An increase in concentration of C reactive protein (CRP), serum amyloid A protein (SAA), and α_1-antichymotrypsin (α_1-AT) may be detected within six to 10 hours; an increase in fibrinogen and α_1-acid glycoprotein (α_1-AGP) concentrations may not occur until 24 to 48 hours after injury.

RESOLUTION OF INFLAMMATION

Following discrete tissue injury such as surgery, CRP and SAA concentrations reach a peak plasma concentration at 48 hours and then fall with a half time of 48 hours. This half time reflects resolution of the injury and is clinically more important than the half life of the protein itself (four to six hours in the case of CRP). Proteins such as fibrinogen and α_1-AGP show protracted half times of 96 to 144 hours.

SENSITIVITY

The incremental increase in CRP and SAA is very much greater (10^2–10^3) than that of any other acute phase protein. These two proteins are therefore the most sensitive to a small inflammatory stimulus. Owing to the wide reference range for CRP (0·1–8·0 mg/l), large increases may occur in a patient while remaining in the normal range. The reference range for SAA is narrower and abnormally increased values are more common. Sequential measurement of these proteins is therefore extremely important for effective monitoring of inflammation in a patient.

CATABOLISM OF PROTEINS

Proteins such as haptoglobin, fibrinogen, and α_1-AT may be actively catabolised if inflammation is accompanied by haemolysis, intravascular coagulation, or vasculitis, respectively. The plasma protein concentration will then be inappropriately low for the extent of inflammation. Such discrepancies can, however, provide useful information on the pathological complications of disease.

Genetic deficiencies of α_1-AT and haptoglobin are common in certain populations, rendering these proteins unsatisfactory as measures of the acute phase response.

DETECTION OF INFLAMMATION

Detection of acute inflammation requires a rapidly responding and sensitive protein such as CRP. Diagnosis of chronic inflammatory disease, however, requires the additional measurement of a slower reacting acute phase protein such as fibrinogen or α_1-AGP, as CRP may be transiently normal when such patients are in short term remission. In this case measurement of the ESR or plasma viscosity, which are sensitive to the effects of multiple proteins, is preferable.

MONITORING OF INFLAMMATION

Monitoring of remission of acute inflammation—for example, response of infection to an antibiotic—is best undertaken by measuring a rapidly reacting acute phase protein such as CRP. In chronic inflammation, however, short term changes in CRP may reflect a transient complication and may thus give misleading information as to the basic disease. For this reason a slower responding test such as the ESR or plasma viscosity is preferable.

LABORATORY MEASUREMENT OF C REACTIVE PROTEIN

Measurement of CRP should be made using a method capable of quantitative measurements down to a serum concentration of at least 8 mg/l. Qualitative or semiquantitative latex agglutination methods are insensitive and of little value. Radial immunodiffusion and electroimmunoassay procedures are relatively slow. Suitable rapid assay methods include laser immunonephelometry or turbidimetry, homogeneous enzyme immunoassay, enzyme linked immunoassay, fluorescein immunoassay, and radioimmunoassay.

An international reference standard for CRP has been accepted by the World Health Organization.[10] Whichever assay method is used, the results should be calibrated against this standard or a secondary standard referable to it; the secondary standard may also be used for quality control.

Conclusion: selection of laboratory tests for monitoring the acute phase response

An increase in blood concentration of acute phase proteins indicates the presence of tissue inflammation whether caused by trauma, ischaemia, infection, or other disease. Laboratory measurement of the acute phase response is of value in: (i) detecting the presence of tissue damage and an inflammatory response; (ii) assessing the size of the inflammatory response, which may be of prognostic value; and (iii) monitoring the response to treatment.

Because a wide range of tissue insults may initiate an acute phase response and because different acute phase proteins show different kinetics, it is to be expected that the nature of the acute phase response will differ in different clinical disorders. Furthermore, in chronic inflammation the response may be complicated by anaemia, a fall in albumin concentration, and an increase in plasma concentration of multiple proteins. It is, therefore, not surprising that there has been controversy over the best laboratory method to quantify the response.

The available methods fall into two main groups—specific assay of individual acute phase proteins and non-specific tests such as the ESR, or other tests of erythrocyte aggregation, and the plasma viscosity. Their respective advantages and disadvantages are shown in the table.

When an acute (less than 24 hours) change in the acute phase response is expected, quantitative assay of CRP is the test of choice. Serum amyloid A protein may be too sensitive, showing a rise in blood concentration within six to 10 hours in response to mild viral infections such as the common cold.

In the later stage of the acute phase response (more than 24 hours) specific assay of fibrinogen or α_1-AGP may give useful information, but in view of the complex nature of the hyperproteinaemia at this stage

Table *Advantages and disadvantages of tests used to assess the acute phase response*

Specific assay of individual acute phase proteins	*Non-specific tests (ESR, plasma viscosity, erythrocyte aggregation)*
Advantages:	
High sensitivity owing to large incremental change (CRP, SAA)	Useful in chronic disease
Fast response (six hours for CRP) to change in disease activity	ESR inexpensive and easy; does not require an electrical power supply
Most proteins can be measured on stored serum	Plasma viscosity has low running costs and the result can be obtained quickly
Automated analysis possible	
Small sample volumes	Plasma viscosity and ZSR not affected by anaemia
Disadvantages:	
More than one protein required to measure acute (CRP) and chronic (fibrinogen, α_1-AGP) inflammation	Not sensitive to acute changes (< 24 hours) and not specific for the acute phase response
Costly when assayed singly or in small batches	Slow to change in response to alteration in disease activity
Need for sophisticated equipment for rapid quantitative assays	Insensitive to small changes in disease activity
Antisera required	Fresh samples (< 2 hours) required for ESR

it is preferable to use a less specific test which is sensitive to the combined effect of several plasma proteins.

Of the non-specific tests, the ESR and plasma viscosity remain the leading contenders. The newer tests of erythrocyte aggregation are of potential value and their development should be encouraged. Laboratory and clinical evaluation of their efficacy will then be required.

The ESR remains the most widely used of these tests despite its recognised disadvantages which include the influence of anaemia, erythrocyte deformability, and immunoglobulins which are not acute phase reactants and may, therefore, blunt the sensitivity of the test. The ESR reference range is significantly affected by age and sex. In some chronic disorders associated with anaemia or hypoalbuminaemia the ESR performs well because both anaemia and a low albumin concentration increase the sedimentation rate. The test is popular because it is inexpensive and easy to perform. Neither centrifugation nor an electrical supply is required so that the test is widely used in developing countries, although the coexistence there of anaemia and haemoglobinopathies limits its value. If a laboratory performs ESR measurements, however, quality control is essential.

Measurement of plasma viscosity has several advantages over the ESR, including independence from the effects of anaemia; a single reference range that is independent of sex and less dependent on age; instruments that can be calibrated; convenience of quality control checks; and results that are available within 15 minutes. The relative unpopularity of plasma viscosity, compared with the ESR, is largely the result of a failure by instrument manufacturers to develop fully automated instruments (including the centrifugation step) that meet current biohazard safety requirements. Development of such instruments is to be encouraged so that the potential benefits of the test can be realised.

Neither the ESR nor plasma viscosity is the test of choice for measuring the short term changes (less than 24 hours) in the acute phase response as both tests are influenced by plasma proteins that are either slowly responding acute phase reactants (such as plasma fibrinogen) or are not acute phase proteins (such as immunoglobulins). Thus measurement of ESR or plasma viscosity is complementary to measurement of CRP and should be used for a different clinical purpose.

Comments on these guidelines are invited and should be addressed to the ICSH Executive Secretary, Dr RL Verwilghen, University Hospital, Herestraat 49, B-3000 Leuven, Belgium.

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