Electrical impedance measurement as an endpoint detection method for routine coagulation tests

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ABSTRACT

A whole-blood platelet aggregometer is adapted to measure electrical impedance changes in plasma during prothrombin time (PT) estimations. The impedance curve shows an acceleration phase, which is comparable to the absorbance curve acceleration phase associated with the onset of coagulation. The amplitude of the impedance change correlates with the fibrinogen concentration of the plasma. Statistical analysis of PT derived by absorbance and impedance changes shows a significant difference between the two methods but a good correlation. The method is reproducible but laborious and requires attention to technique. Further investigation of the method utilising a more sensitive instrument and redesigned electrodes is indicated. It may also be possible to modify reagent systems to optimise impedance changes.

KEY WORDS: Blood coagulation.
Impedance.
Prothrombin.
Prothrombin time.

Introduction

Routine coagulation tests are designed to provide rapid, relatively non-specific information on the general nature of an abnormality and direct the investigator to the analysis of discrete coagulation factors and hence a diagnosis.[1] The techniques normally used to screen for haemostatic defects are prothrombin time (PT),[2] partial thromboplastin time (PTT),[3] and thrombin clotting time (TCT).[4,5] PT is also used widely to monitor the treatment of patients receiving oral anticoagulant therapy.[6]

With increasing workload, there have been many developments in the automation of these tests,[7] and this has had two main areas of focus: the sample and reagent handling aspects of the tests and the endpoint determination.[8]

The routine coagulation tests described above rely on measurement of the time elapsed from initiation of the coagulation process to the onset of fibrin clot formation. The endpoint is detection of fibrin strands in the detection mixture or the gelling of the reaction mixture prior to the formation of visible fibrin strands.[7]

Automating the sample handling aspects of these tests helps to eliminate the variables (e.g. temperature, evaporation, calibration of pipettes and accuracy of timing) that affect accuracy, precision and reproducibility. Automation of endpoint detection has seen different manufacturers develop analysers with detection methods that either mechanically detect clot formation or optically detect clot formation (Table 1).

Table 1. Examples of endpoint detection methods used in automated coagulation analysers

Manufacturer and Analyser	Principle of detection method
Hyland, Clotek	Movement cessation of ball bearing
Sarstedt, Biomatic	Viscosity change
Nygaard, Thrombotrak	Movement cessation of ball bearing
General Diagnostics, Coag-A-Pet	Absorbance change
Thrombolytic Assessment System	Movement cessation of iron particles
Instrumentation Laboratory, ACL	Absorbance change

Application of the tests in near-patient testing (NPT) has seen a great deal of interest recently, especially in the area of anticoagulation monitoring. Current automated techniques are providing benefits for NPT but are proving expensive in terms of reagents or equipment, and some techniques require close attention to detail to obtain accurate results.[9]

An alternative method for endpoint detection of coagulation tests is measurement of electrical impedance, which has been used previously to study whole-blood clotting times and clot retraction.[10-12]

This study evaluates electrical impedance measurement to determine the endpoint of coagulation tests and compares the results with a widely used automated technique.

Materials and methods

The measuring device used was a Chronolog model 540 dual-channel platelet aggregometer (Chronolog Corporation, Havertown, PA, USA) capable of measuring changes in absorbance (*A*) and electrical impedance simultaneously. The *A* channel operates in the infrared

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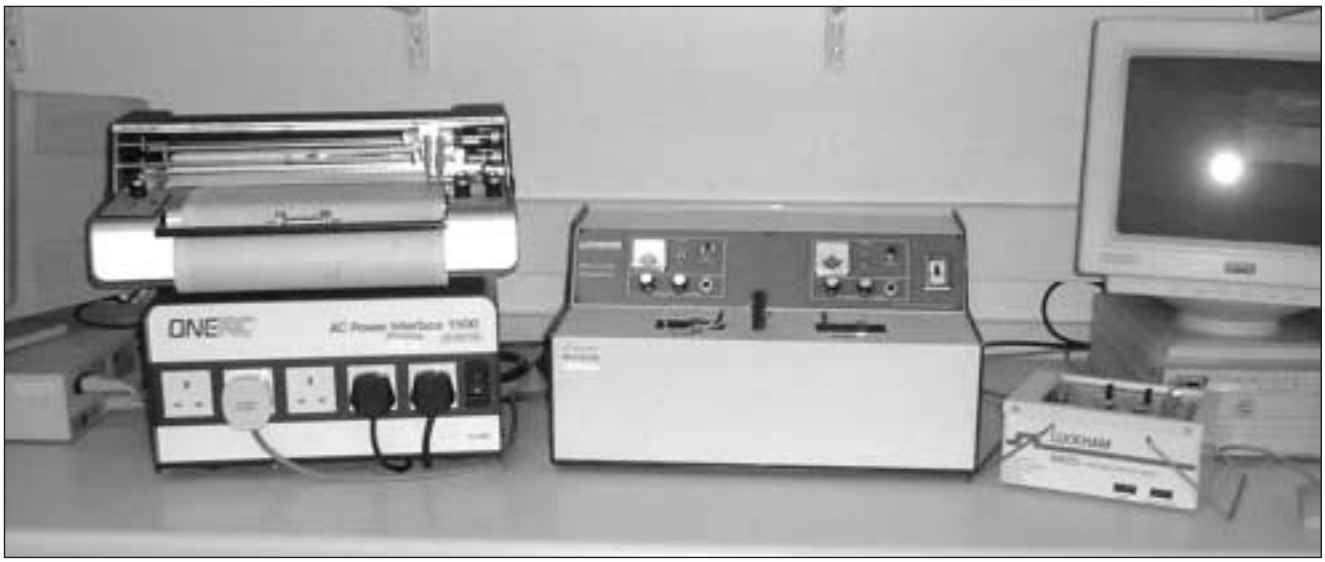


Fig. 1. Photograph showing equipment used, with the Chronolog 540 aggregometer (centre), stabilised power supply with chart recorder on top (left) and a water bath for pre-incubating samples, reagents and probes (right).

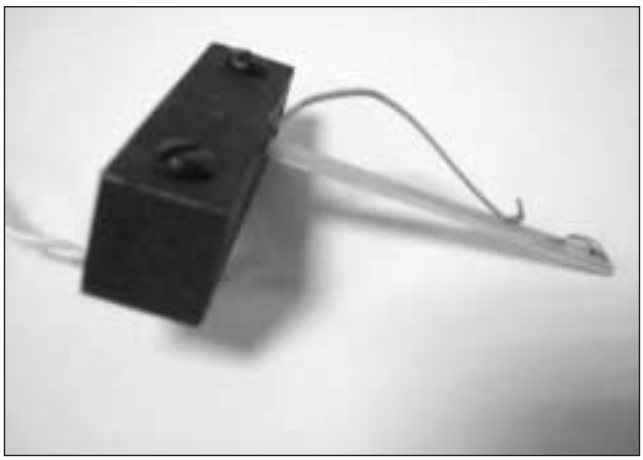


Fig. 2. Close up view of impedance probe assembly showing the electrodes on the extreme right of the picture.

spectrum (650 nm) and features an automatic baseline and gain setting which configures the machine to measure reduction in *A* relative to a reference standard.

The electrical impedance channel utilised a 15 kHz sine wave signal with a peak-to-peak amplitude of 100 mV. The output from each channel was connected to a Houston Omniscribe dual-channel strip chart recorder. To prevent spurious electrical interference, both instruments were connected to the mains electrical supply via an ONEAC high-efficiency voltage stabiliser and electronic noise filter (ONEAC Europe, Abingdon, Oxfordshire OX14 1TR, UK).

The impedance sensor probes were re-usable printed circuit devices consisting of an insulated plastic support on which was mounted an electrode assembly consisting of two semicircular lengths of platinum wire, approximately 5 mm long with a gap of 0.5 mm. The assembly was constructed to allow insertion into the measuring cuvettes to the same position each time. The measuring cuvettes were siliconised flat-bottomed glass tubes with an internal diameter of 10 mm (Figures 1 and 2).

Impedance measurements

Chart recorder input sensitivity was set to 100 mV. The zero position of the chart recorder pen was adjusted while applying a zero voltage from the Chronolog 540 to the input of the recorder. Then, with the impedance probe in position, the pen was returned to the zero position by adjusting the output from the Chronolog 540.

Output gain was then adjusted to give a suitable pen deflection to a 5-Ohm (Ω) calibration pulse that was recorded on the chart recorder. On addition of plasma, the zero position altered due to change in overall impedance, and the pen was returned to the zero position by manual adjustment.

Optical measurements

Chart recorder input sensitivity was set to 100 mV. The zero position of the chart recorder pen was set while applying a zero voltage from the Chronolog 540 to the input of the recorder.

Zero position and gain were set automatically by a 'set baselines' function on the Chronolog 540. This automatic function configured the machine to measure decreasing *A*; therefore, to determine increasing *A* during coagulation the positions of the reference standard and reaction mixtures were reversed. On addition of plasma, the zero position altered due to change in *A* and the pen was returned to the zero position by manual adjustment.

Optical measurements were made relative to a silicone emulsion nephelometric standard. (Instrumentation Laboratory Reference Emulsion Cat No. 97569-00).

For PT estimations 1 mL premixed thromboplastin and calcium chloride reagent (PT-FIB HS; Instrumentation Laboratories) was incubated in the Chronolog 540 to reach thermal equilibrium, 0.5 mL prewarmed plasma was added and the *A* and/or impedance changes were recorded on the strip chart recorder.

Data reduction was performed manually by examination of the traces to determine points where changes in *A* or impedance occurred. Impedance values were calculated relative to the calibration pulse performed with each test.

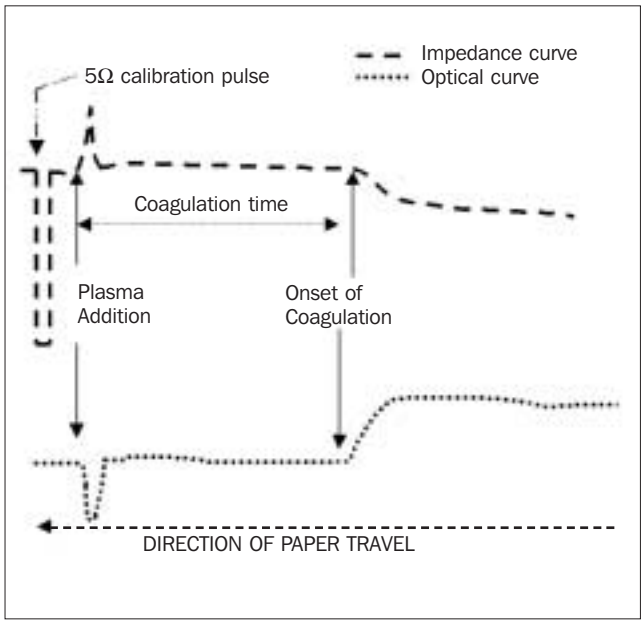


Fig. 3. Impedance and optical coagulation curves.

Coagulation times were calculated from the length of trace and the speed of paper. Samples were also analysed on a Futura analyser (Instrumentation Laboratories) for reference purposes.

Statistical analysis was performed using Minitab software.

Results

The stability of the test system was confirmed by measuring impedance and *A* on reagent and plasma samples over a 10-min period. Early trials indicated significant fluctuations in impedance from initial baseline settings due to drift of the mains voltage supply producing plateau changes and electrical 'noise' from nearby thermostatically controlled equipment producing spikes on the baseline. Introduction of a power supply filter minimised mains voltage fluctuations and filtered out the voltage spikes caused by other equipment.

Subsequent stability runs showed no significant positive or negative deviations from baseline settings, with deviation of ≤ 0.1 Ω. Absorbance measurements were not influenced by voltage drift or spikes and remained stable throughout the stability trials.

Prothrombin time estimations were performed on 20 replicate samples of lyophilised human plasma (Technoclone abnormal control plasma; Technoclone, Dorking, Surrey RH4 1EJ, UK) to compare curve shape and reproducibility. A typical curve comparison is shown in Figure 3. The optical curve shows a typical 'lazy S' shape of an initial baseline phase followed by a steep acceleration phase and a final horizontal endpoint. The impedance curve showed a similar baseline phase followed by less well-defined acceleration phase and a final slowly increasing phase.

Impedance changes were in the range 0.8 to 1.3 Ω (mean: 1.08 Ω, standard deviation [SD]: 0.14). Fibrinogen concentration and impedance change to onset of coagulation on the same set of samples showed a Pearson correlation of 0.701 ($P<0.005$; Figure 4)

Coagulation time is defined as the time from addition of plasma to the start of the acceleration phase and was calculated from trace length and chart speed. There was a significant difference in times derived from the optical curves and those derived from the impedance curves ($P<0.001$ by paired *t*-test): optically derived times were shorter than impedance times (Figure 5).

Prothrombin time was performed on 34 patients receiving oral anticoagulant therapy using the impedance and optical methods. The Pearson correlation coefficient for the data

Fig. 4. Linear regression of impedance change and fibrinogen concentration.

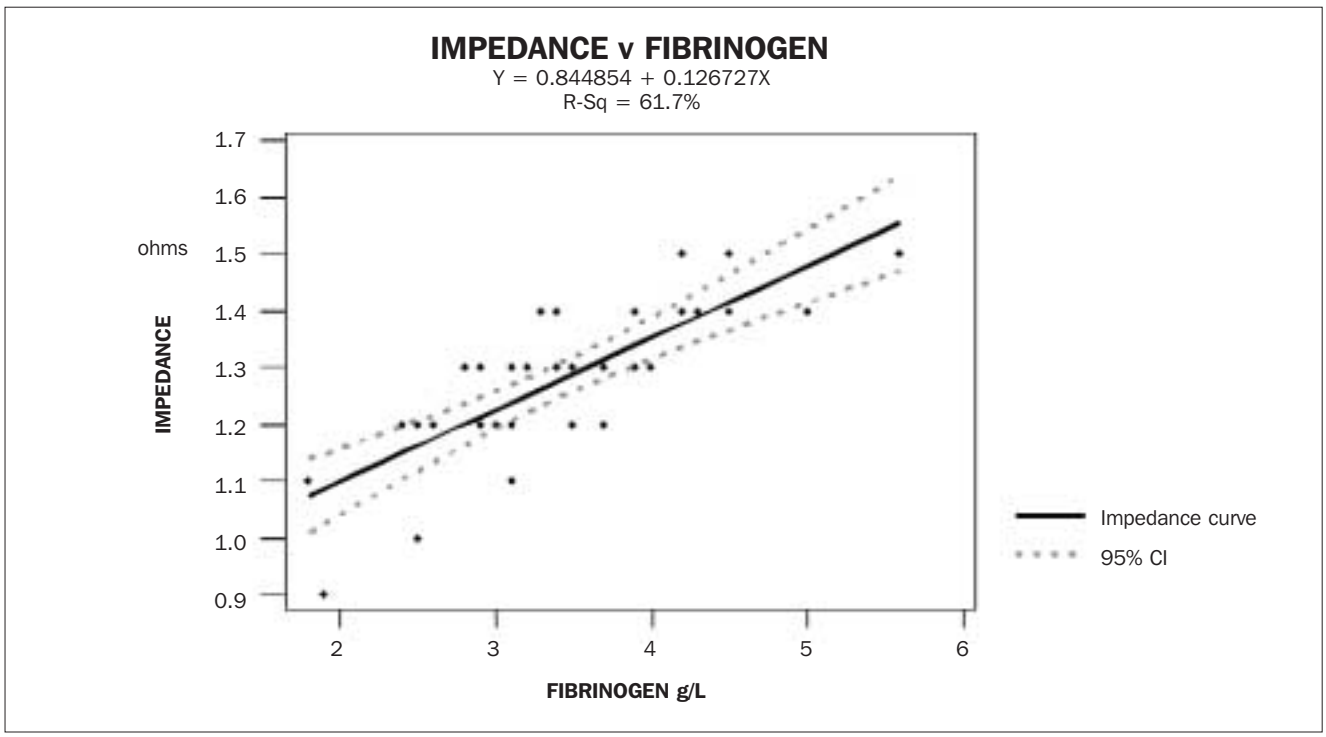
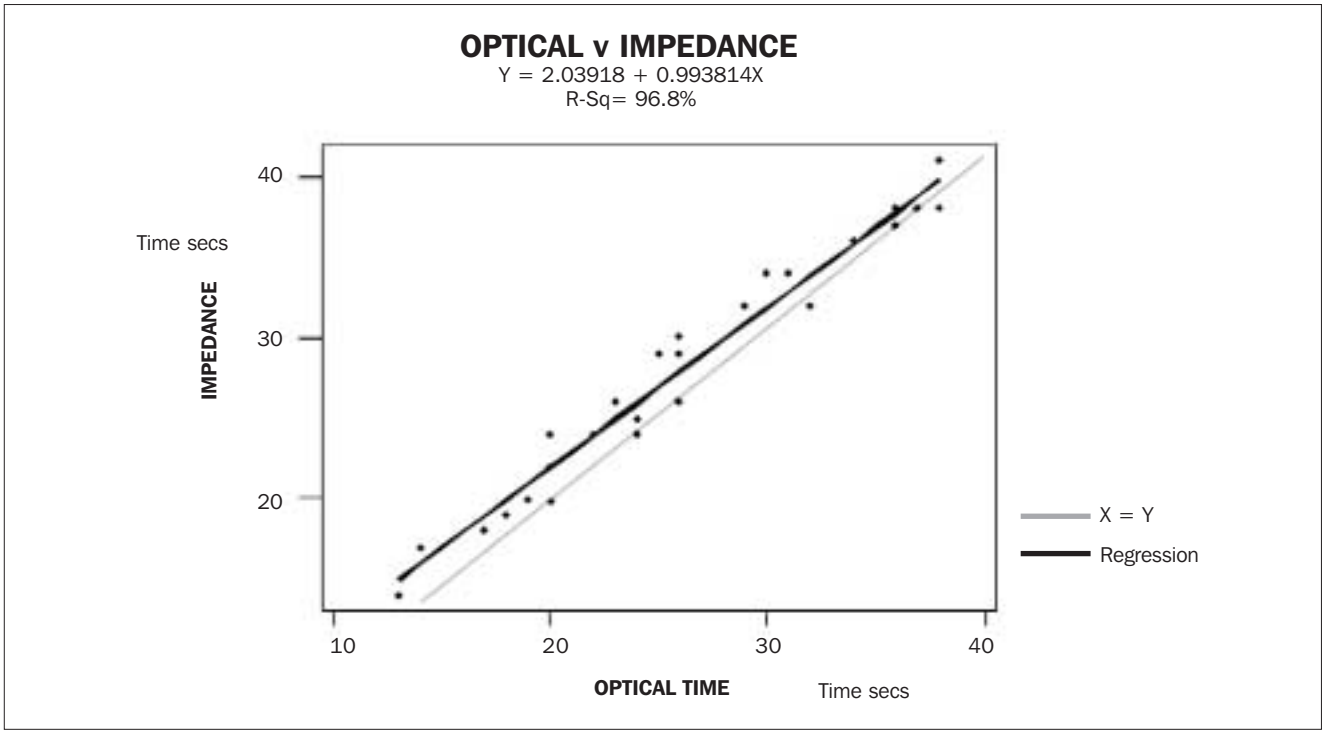


Fig. 5. Linear regression of optical and impedance-derived PT times.



was 0.985 ($P<0.005$) and a paired Student's *t*-test showed a significant difference in the two sets of data ($P<0.005$). Comparison of PT times derived by the impedance method and those from the Futura analyser showed a Pearson correlation of 0.972 ($P< 0.005$; Figure 6).

Discussion

Measurement of impedance changes during blood coagulation requires close control of the conditions that affect the electrical conductivity of blood.[13] Temperature control is critical as the electrical impedance of whole blood has a temperature coefficient of around 1.5 %/°C.[14] Use of an alternating current (AC) signal source is essential to prevent polarisation and allow the simultaneous measurement of the capacitive reactance and resistance that together form the overall impedance measurement.[15] High voltages may cause heating of the sample and this can lead to temperature-induced impedance drift. Finally, the size and geometric arrangement of the electrodes is important as these determine the magnitude of the impedance measured.[16]

Within the restrictions imposed by the available apparatus, all these conditions were adequately controlled for the purposes of the study. Temperature was automatically controlled within 0.1°C by the Chronolog 540, and the AC signal voltage and frequency (100 mV, 15 kHz) did not produce any appreciable temperature-induced impedance changes. As the impedance changes being measured were very small and the apparatus was operating at the limit of its sensitivity at near maximum gain settings, the use of a stabilised and filtered power supply was essential. Previous work has also identified the stability of the measuring system as a critical factor in impedance measurement of blood coagulation.[17]

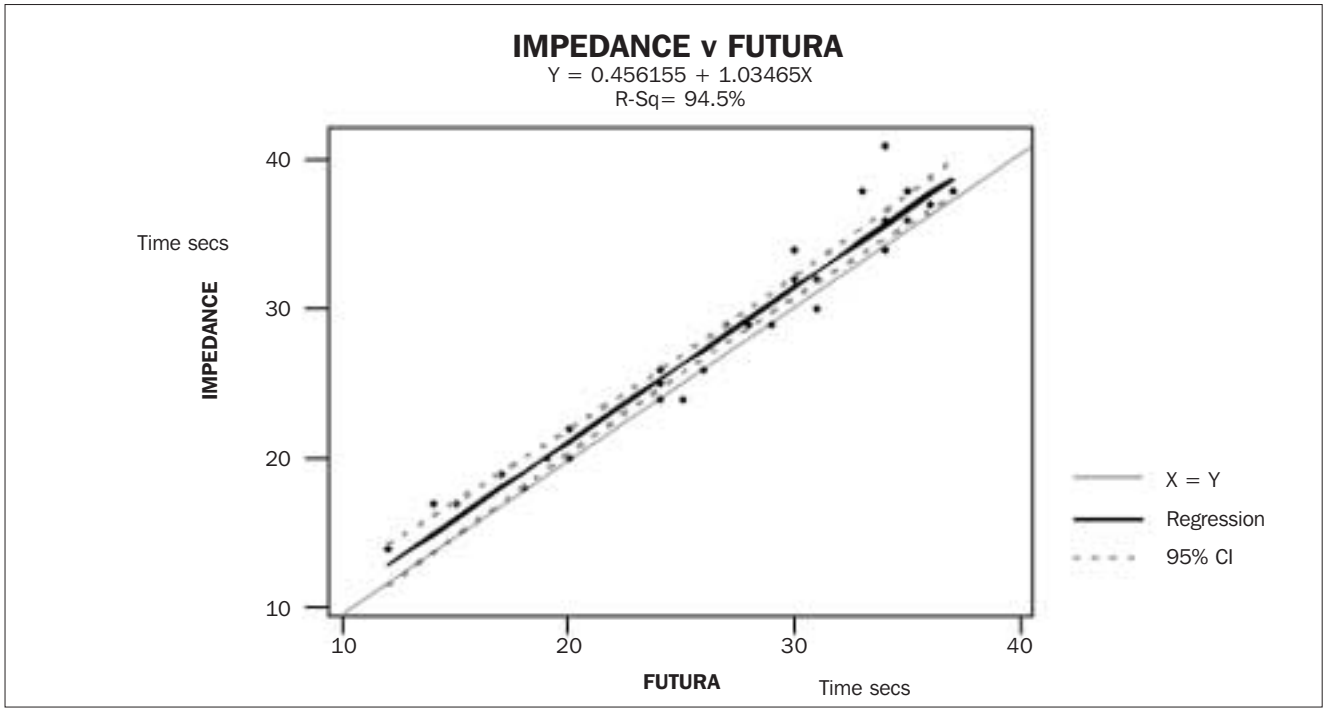
Comparison of the impedance and optical curves showed that the acceleration phase of the impedance curve lags behind the optical curve by a few seconds. The reason for this is unclear but may be explained by the way in which fibrin causes the physical changes detected by the two methods.

Fibrinogen is distributed evenly throughout the reaction mixture and precipitates as fibrin in a homogeneous manner throughout the mixture during coagulation, causing a change in *A*.[18] For a change in impedance to occur, however, fibrin must build up on the surface of the electrode to form an insulating layer.[16] Clearly, this will take some time to occur and could explain the delay in the acceleration phase of the impedance curve. The significance of the slow increase in the impedance curve that follows the acceleration phase is unclear but may be due to cross-linking of fibrin polymers.

Both methods rely on the conversion of fibrinogen to fibrin to detect the onset of coagulation and therefore rely on sufficient levels being present to produce a detectable change. The impedance method reliably detected changes in fibrinogen level down to 1.8 g/L in the group of patients tested. However, although the size of impedance change correlated with fibrinogen concentration (Figure 5), it was not sufficient to predict fibrinogen levels. This poor correlation could be due to the lack of sensitivity in the current detection system.

Although there was a significant difference in the means of the optical and impedance groups, due to the delay in the acceleration phase, there was very good correlation between them (Figure 6). Comparison of the impedance-derived times with the routine laboratory method also showed good correlation. The difference between the means of the optical and impedance results can be overcome by reporting results relative to a control value or normal range derived by the same method – which is standard laboratory practice.[19]

Fig. 6. Linear regression of impedance-derived PT time and PT time by IL Futura analyser.



Electrical impedance is capable of detecting the endpoint of coagulation tests and correlates with current methods; however, further work is required to develop the method. Some current problems are probably related to the detection system, which was adapted from another use. A purpose-built detection system would be microprocessor-controlled with automatic baseline, range and sensitivity settings to overcome variations in sample conductivity. Re-usable electrodes would need to be replaced by a disposable device – with optimised electrode size and geometry – that could be mass-produced and possibly have the reagent system included, as with blood glucose testing systems. It might also be possible to alter the reagent system to incorporate substances such as latex particles to enhance the impedance change and produce an endpoint closer to the optical reference system.

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