THE SUCCESSFUL HISTORY OF THE AUTOMATED COAGULATION LABORATORY (ACL)

Armando Tripodi

Full Professor of Laboratory Medicine, Humanitas University
Established Investigator, Angelo Bianchi Bonomi
Hemophilia and Thrombosis Center, Milano, Italy

The fascinating history of hemostasis testing started more than one century ago and has been the focus of one of my previous publications[1].
In this manuscript, I examine the interesting history of how the revolutionary concept of the Automated Coagulation Laboratory (ACL) instrument came about.

Index

Manual tilt tube (or hook) technique 4

Earlier coagulometers 6

Photo optical coagulometers 7

The study 7

Centrifugal analyzers 9

Coagulation testing based on centrifugal analyzers 10

What's next 14

Acknowledgements 15

Manual tilt tube (or hook) technique

Modern laboratory operators, currently engaged in measuring coagulation parameters, can hardly imagine that until the late 1970's coagulation testing was mainly performed by manual (tilt tube or hook) technique by skilled operators (Fig 1), who could manage to handle glass tubes immersed in a water-bath at 37°C. After dispensing plasma and activators into the tubes, a stopwatch was started and the tube(s) tilted back and forth at regular interval until the clot formed. Operators were trained to see the first strand of fibrin and stop the stopwatch concurrently. The reproducibility (imprecision) of this procedure when assessed as the coefficient of variation on repeated measurements of the same sample was not that bad, although not comparable to that achieved by modern coagulometers. I also recall some operators in our lab, who were so skilled to handle three tubes simultaneously (Fig 2), when they performed the measurement of factor VIII activity for which at least three different (increasing) plasma dilutions were used to perform a bioassay by means of parallel lines (patient and standard plasma). Obviously, during the procedure, the operators had to handle not only three tubes, but also to look at three stopwatches simultaneously. They were however facilitated by the fact that, owing to the increasing plasma dilutions in the three tubes, the clotting times were also increasingly longer and therefore their exact appreciation was relatively simple.

Figure 1



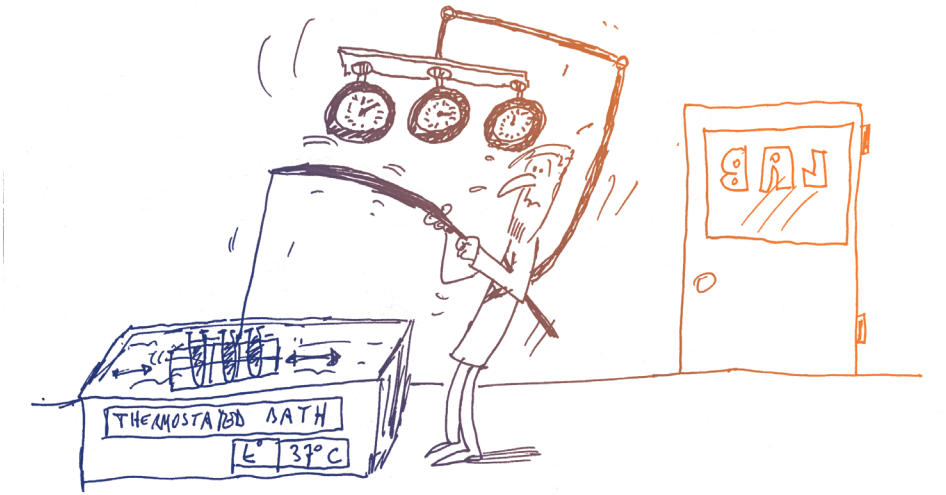


Figure 2

Vijay Parekh, a long lasting friend of mine, came from Mumbay (India) to our lab in the late 1970's for a training fellowship. He was so adamantly keen to get unbiased results that he put his three stopwatches with their clock face hidden. He stopped them as soon as the clot started to form without looking at the clotting times they had recorded (Fig 3)



Figure 3

Earlier coagulometers

Although this was the state of the art, in the late 1970's coagulometers started to appear in my lab.
They have been implemented following Mannucci's experience at the Oxford laboratory for hemophilia and allied disorders in the UK that was at that time one of the most important in the World. Those coagulometers had an electromechanical system of clot detection based on two platinum electrodes that moved within the mixture of plasma and reagents. When the impedance measured by the electrodes started to change because of clot formation, the movement of the electrodes (as well as the timer) stopped and the clotting time was recorded. Cuvettes used for the reaction were disposable, but the electrodes could not be changed at each test occasion.
To avoid thrombin carry over and contamination from one sample to the next, the electrodes had to be flamed from one sample to the next in the series of measurement (Fig 4).

Figure 4

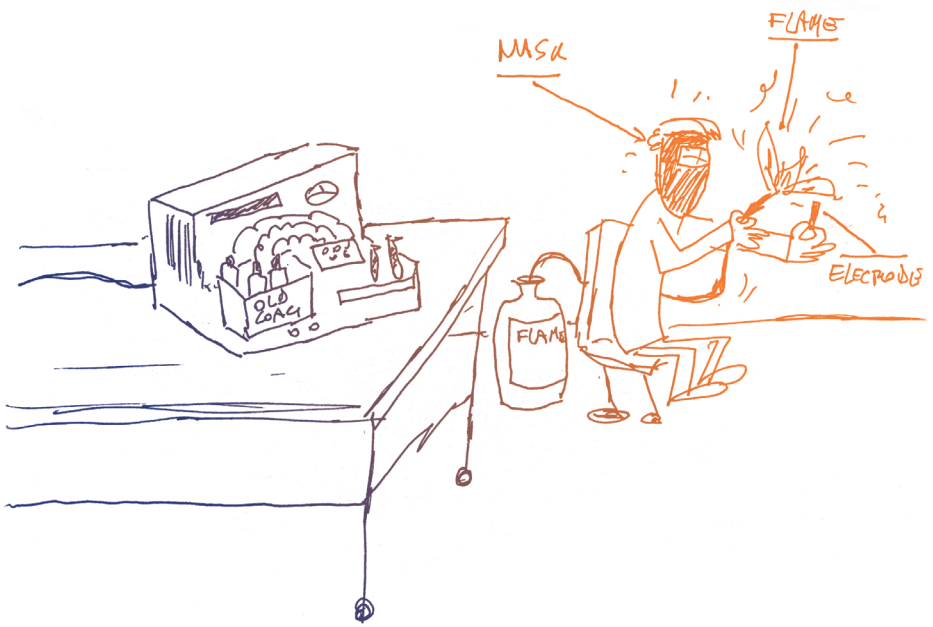


Photo optical coagulometers

Early electromechanical systems were soon replaced by photo optical clot detection systems installed in the coagulometers, which were also capable of pipetting plasma and reagents into the reaction cuvette. Among them, I remember the Coagulazyer Lancer II and CoagaMate X2, which operated in our lab for many years during the late 1970's and the early 1980's. Despite the evident advantage brought about by the coagulometers, we were however not fully convinced that the ability of the coagulometer to detect clot formation was superior to that of a trained lab operator using the manual (tilt tube) technique.

The study

A seminal study was organized within the frame of activity of the Italian Committee for Standardization in Laboratory Hematology (CISMEL) to which I was affiliated. The study was designed to answer the question of whether coagulometers were better than the manual (tilt tube) technique run by an expert lab operator[2]. That question, which may now be regarded as a nuisance, had at that time some value and required evidence stemming from a trial. Nine brands of the coagulometers that were on the market at that time were taken to the labs and operated by expert technicians provided by the respective manufacturers. These operators ensured that the coagulometers worked at the best of their ability, thus avoiding any possible bias. The samples used for testing were a set of coded freeze-dried plasmas prepared with a special design unknown to the participants. One subset of the plasmas was at increasing prothrombin time (PT), starting from normal to high values. The second subset was prepared to get paired PT that were similar, but not identical to the PT of the first subset. This special design provided (within each set) couples of plasmas with similar, but not identical PT. Therefore, coagulometers and lab operators were compared for the ability to distinguish the plasmas with similar, but not identical PT. The data stemming from the study within each comparison were analyzed for statistical differences by testing the mean value obtained for each plasma subset by the coagulometers vs lab operators. The test used was the "Student t"; by definition, the greatest the "t" value stemming from the statistical analysis, the better the ability of the method (either the coagulometer or the lab operator) to distinguish the two samples at similar, but not identical PT. These results were taken as indexes of the reproducibility of the clot detection systems under investigation. I still remember the excitement when the operators of the commercial companies that accepted to participate in the study brought their coagulometers in to the labs and the reconstituted sets of plasmas were given to them and to the expert (manual) operators, who worked at the same time in the same room.

When results were analyzed, it was evident that coagulometers won the game in many instances, but not in all of them (Table 1). Some manual operators performed better than the coagulometers, thus leaving room for further improvement. Nonetheless, the above exercise gave support to the use of coagulometers and many labs started to switch from the manual (tilt tube) technique to the more sophisticated clot detection systems.

Table 1

"t" values for the comparison of manual vs coagulometers. The higher the "t" value the better the capacity of the clot detection system to discriminate between two plasma samples with similar but not identical PT value. Adapted from Italian Cismel Study Group, Clin Lab Haematol 1983; 5: 177 with the permission of the authors.

Methods of clot detection	Coagulometers								
	1	2	3	4	5	6	7	8	9
Manual	0.45	8.60	5.67	5.74	6.51	3.60	5.82	8.69	2.39
Coagulometer	4.79	4.97	5.99	2.74	7.88	11.58	5.58	6.56	6.07

Coagulometers:

1 Coag-A-Pet (General Diagnostic)
2 Schnitger-Gross (Amelung)
3 Multiclot 919 (Elvi)
4 Multiscan 818 (Elvi)
5 Coagulazyer Lancer II (Sherwood Medical)
6 Fibrintimer II C (Labor).
7 Electra 600 (Medical Laboratory Automation)
8 Sistema Coagg Salus (Daltec Instrument)
9 Coagulation Profiler CP-8 (Biodata Corporation)

Centrifugal analyzers

Instrumentation Laboratory (IL), whose headquarter and plant were at that time in Italy, had a great experience in testing clinical chemistry parameters by means of auto analyzers based on a new (revolutionary) system developed to be used even in the absence of gravity such as the one operating during space missions. The system was based on a multi-cuvette plastic rotor in which there were three compartments: the first for the patient sample; the second for the reagents and the third was the reaction cuvette (Fig 5).

Figure 5



The three compartments were separated by two bumps to avoid sample and reagents to come into contact before starting the analysis.

An automated pipetting system consisting of two probes delivered samples and reagents into the respective compartments within the rotor. A rinsing system minimized the carry over and contamination between samples. After samples and reagents were delivered, the rotor was set in motion and the ensuing centrifugal force allowed samples and reagents to overcome the bumps between the three compartments and to mix at the bottom of the cuvette rotor where the reaction took place.

A sophisticated photo optical system recorded the optical density in each cuvette, which was thereafter translated into the analyte concentration by reference to a set of standards at known concentrations, which were concurrently placed in specific positions within the same rotor [3]. Despite (or thanks to) the mechanical sophistication, the system was very reliable and easy to be run even by poorly trained operators. It gained rapid acceptance in many labs and competed successfully with the existing clinical chemistry auto analyzers that were based on flowing systems in which samples from individual patients were separated by air bubbles to avoid cross contamination [4].

Coagulation testing based on centrifugal analyzers

Research and development at the IL plant was at that time managed by Claudio Calzi, who decided to appoint a group of research workers to see whether the concept of centrifugal analysis could be applied also to coagulation. The initial team consisted of two researchers, Giovanni Ceriani and Luigi Preda.
The first (an engineer) was responsible for the mechanical/electronic development and the second (a biologist) for the methods set up. They did however have little expertise in the field of coagulation and therefore C. Calzi came to my boss (P. M. Mannucci), who was one of the most prominent opinion leader for hemostasis in Italy and asked for advice. Mannucci realized the importance of the project, but declined a direct participation and appointed myself to help the team. At that time, I was at the beginning of my career and was very keen to be part of that exciting project. We started a series of meetings devoted to discuss the main coagulation assays where the new coagulometer could be useful for the clinical laboratory. At that time most of the assays were clot-based, few were based on immunochemistry and very few on synthetic chromogenic substrates. After exclusion of the immunoassays, which were outside the capacity of a clot-based detection system, we soon realized that the new coagulometer could handle clot-based parameters such as PT and activated partial thromboplastin time (APTT) and congener tests such as thrombin clotting time and individual coagulation factor measurement. However, what about the antithrombin activity?

Figure 6



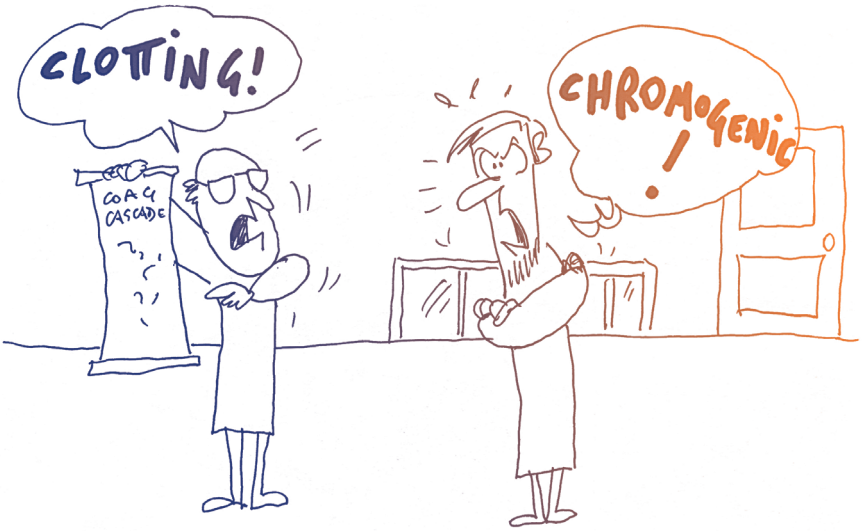
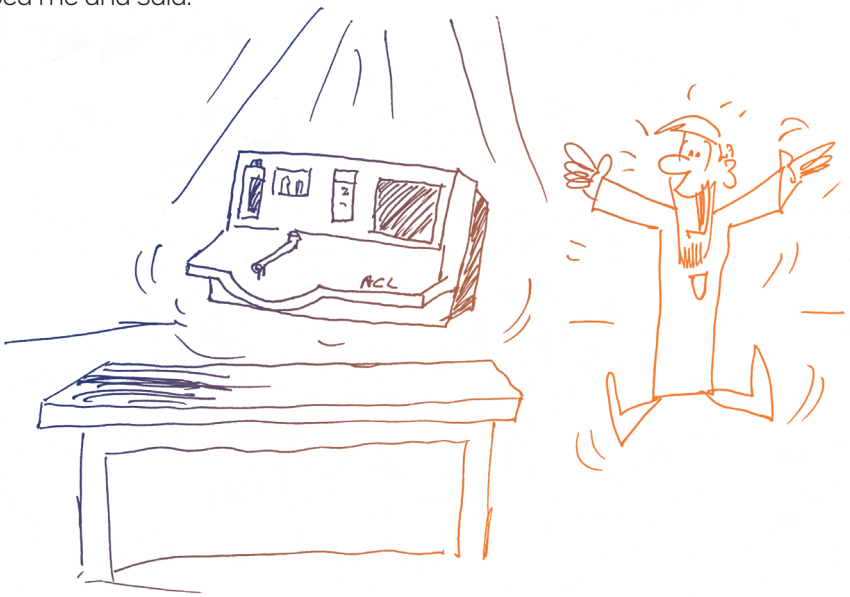


Figure 7

That measurement was emerging after the report by Egberg et al. [5], who described the first family, whose members were affected by an inherited deficiency of that naturally occurring anticoagulant and had venous thromboembolism. The concept of 'thrombophilia', which was still unknown, started to emerge and to parallel the concept of "hemophilia". Many clinicians were excited and wanted to look for antithrombin deficiency in their patients with venous thromboembolism. Everybody wants to measure antithrombin, but the method described by Egberg et al. was based on a synthetic chromogenic substrate for which the endpoint was optical density, not clot formation. Readings for that method would have needed a photo optical system capable of measuring optical density at 405 nm, which was however far away from the wavelength commonly used to detect clot formation. Hence, antithrombin measurement could not be performed on any of the existing coagulometers and a photometer had to be used instead. I remember that we discussed for several weeks on how to resolve that issue (Fig 6). Our main concern was that with this scenario, the coagulation laboratory would have had to use two types of instruments (one clot-based and one optical density-based). L. Preda and I were in sharp contrast. While he was strongly convinced that antithrombin could have been successfully measured by using a clot-based method, I supported the idea of the chromogenic substrate (Fig 7). At that time, I was strongly convinced that chromogenic substrates would have eventually replaced clot-based systems.

My belief was stemming (at least in part) from the fact that I and others in the field considered the chromogenic substrate technology more reliable than coagulation in terms of reproducibility. In agreement with this belief, many research workers within the commercial companies started to develop the basic tests PT and APTT, which were based on chromogenic substrates and could therefore be set up on clinical chemistry auto analyzers [6, 7]. According to Dr. Stormorken, a former Scandinavian laboratory worker, 'bells were ringing' for water-bath and stopwatches because of the upcoming coagulation testing with chromogenic substrates. The dream of the clinical chemists had come true: coagulation factors could have been measured as ordinary "enzymes" and their results expressed in terms of "catalytic units" and not as "percent activity" (Fig 8).
I must confess that my perception was (at least in part) wrong: chromogenic substrates technology did not keep entirely their promise. G. Ceriani, who was an engineer with scarce knowledge about coagulation, was puzzled by the fierce contention that was going on between L. Preda and myself on antithrombin measurement. We were not able to reach an agreement and there was a stalemate, which carried the risk of stopping or delaying the project. After a few weeks from our last team meeting, G. Ceriani came to my office alone and said. "Are you really convinced that antithrombin measurement should be performed with the chromogenic substrate technology?"
I promptly answered "yes" and started to provide once again arguments in favor of my belief.
He suddenly stopped me and said.

Figure 8



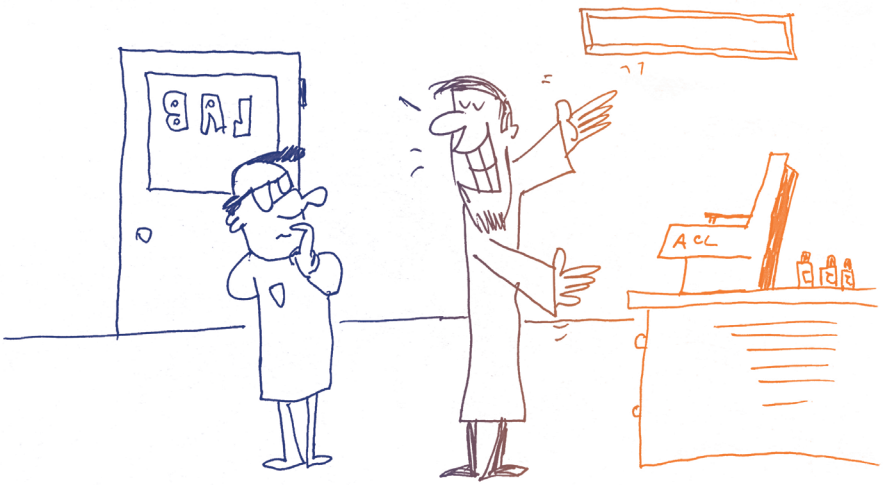


Figure 9

"What do you think if I will develop an instrument having on board of the same unit both a clot-based and a chromogenic detection system?" Unbelievable as it may seem, the solution was relatively simple, but nobody (except for G. Ceriani) promptly saw it. A "chimera" was developed and the ACL was born (Fig 9)! The ACL was a revolution and was instrumental in changing the concept of how coagulation was measured. Met initially with some skepticism, the ACL was soon accepted even in the USA (the most demanding market, especially for product not developed within the country) and spread eventually across the World (Fig 10).



Figure 10

Few years later, all the other commercial companies developed their own units bearing on board both clotting and chromogenic systems. My lab was among the first to evaluate the first ACL prototype before commercialization and the report stemming from that evaluation was published in Clinical Chemistry [8]. G. Ceriani and L. Preda (the latter passed away years ago) must be credited for that success that paved the way to the current measurement of coagulation. I wish also to remind the work of Ernesto Botta, who was in charge of developing in house the early thromboplastins and cephalines to be used in combination with the ACL. This is the story of how a successful project was successfully accomplished by a successful team.

What's next

The story of coagulation measurement started in the late 1800,s when the founding fathers were making earlier observations on whole blood left on the bench to clot in glass tubes. From those crude assays they soon moved to blood centrifugation aimed at removing blood cells and platelets, which were considered not useful for coagulation (blood cells) or difficult to standardize (platelets). The era of coagulation tests on plasma was started and over the subsequent decades, many refinements aimed at standardization of the assays were carried out (see ref n. 1 for more details).
It is now clear that plasma measurements (at least for global tests) do not entirely represent hemostasis as it occurs in vivo where not only plasma, but also other essential components such as red blood cells [9], monocytes, platelets and microparticles (the latter stemming from parent cells that possess procoagulants substances) play a crucial role in the hemostatic process [10, 11]. It is therefore logical to assume that global testing with PT and APTT on whole blood is much more representative of the in vivo hemostasis than testing on plasma. One of the reasons why plasma succeeded over whole blood in the past was the lack of appropriate technology that prevented clot formation to be detected by the optical systems of the coagulometers. Technology is now changed and the next generation of coagulometers could be easily adapted to work on whole blood. Perhaps a team of brave research & development workers such as G. Ceriani and L. Preda would one day make my dream come true.

Acknowledgements

The author wishes to thank Davide Colombo, who contributed with his beautiful sketches to illustrate this history. The author wishes also to mention the following, who did not take part directly in the project, but contributed to disseminate it worldwide: (in alphabetic order) R. Benet (USA), V. Bernardelli (Italy), A. Berti (Italy), L. Bevilacqua (Italy), R. Bottenus (USA), T. Casas (Spain), V. Casadevall (Spain), D. Colombo (Italy), F. Duran (Spain), E Ferretti (Italy), R. Galli (Italy), M Graça (Mexico), S. Gonzalez (Uruguay), E. Lattuada (Italy), J. Manent (Spain), C. Pascual (Spain), M. Piai (Italy), R. Playdon (UK), F. Raimondo (Italy), A. Rigual (Uruguay), L. Rodighiero (Italy), J.M. Rubiralta (Spain), G. Russi (USA), D. Santo (Italy), V. Sarquella (Spain), G. Semprini (Italy), J. Serra (Spain), M. Spagnotto (Italy), A. Strada (Italy), R. Tazzi (USA), G. Tong (China), A. Tonsi (Italy), M. Triscott (USA), J. Llorens (Spain), P. Zamora (Spain).

References

1. Tripodi A. The history of phenotypic testing in thrombosis and hemostasis. Sem Thromb Hemost 2008; 34: 585.
2. Italian CISMEL Study Group (Ciavarella N, Eder G, Mannucci PM, Mariani G, Mori GP, North WRS, Palareti G, Tripodi A. Multicenter comparison of nine coagulometers and manual tilt-tube methods for prothrombin time performance. Clin Lab Haematol 1983; 5: 177.
3. Pesce MA, Centrifugal Analyzers-A New Concept in Automation for the Clinical Chemistry Laboratory. J Chem Edu 1974; 11: A521.
4. Rulika J; Hansen E. H. Flow injection analyses: I. New concept of fast continuous-flow analysis". Anal Chim Acta 1975; 78: 145.
5. Egeberg O. Inherited antithrombin deficiency causing thrombophilia. Thromb Diath Haemorrh 1965; 13: 516.
6. Tripodi A, Mannucci PM. Clinical evaluation of a fully automated chromogenic method for prothrombin time compared with a conventional coagulation method. Clin Chem 1984; 30: 1392.
7. Tripodi A, Poggio M, Scapellato L, Mannucci PM. Automated amidolytic method for activated partial thromboplastin time compared with a conventional coagulation method. Haemostasis 1989; 19: 205.
8. Chantarangkul V, Tripodi A, Mannucci PM. Evaluation of a fully automated centrifugal analyzer for performance of hemostasis tests. Clin Chem 1987; 33: 1888.
9. Du VX, Huskens D, Maas C, Al Dieri R, de Groot PG, de Laat B. New insights into the role of erythrocytes in thrombus formation. Semin Thromb Hemost 2014; 40: 72.
10. Davizon P, Lopez JA Microparticles and thrombotic disease. Curr Opin Hematol 2009; 16: 334.
11. Burnier L, Fontana P, Kwak BR, Angelillo-Scherrer A. Cell-derived microparticles in haemostasis and vascular medicine. Thromb Haemost 2009; 101:439.

Realized in collaboration with

werfen

p/n 98088-63 Rev. 1 - All rights reserved. 07/23