Evolution of Hemostasis Testing: A Personal Reflection Covering over 40 Years of History*

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Abstract

There is no certainty in change, other than change is certain. As *Seminars in Thrombosis and Hemostasis* celebrates 50 years of publication, I felt it appropriate to reflect on my own 40-year plus scientific career. My career in the thrombosis and hemostasis field did not start until 1987, but the subsequent 35 years reflected a period of significant change in associated disease diagnostics. I started in the Westmead Hospital "coagulation laboratory" when staff were still performing manual clotting tests, using stopwatches, pipettes, test tubes, and a water bath, which we transported to the hospital outpatient department to run our weekly warfarin clinic. Several hemostasis instruments have come and gone, including the Coag-A-Mate X2, the ACL-300R, the MDA-180, the BCS XP, and several StaR Evolution analyzers. Some instruments remain, including the PFA-100, PFA-200, the AggRAM, the CS-5100, an AcuStar, a Hydrasys gel system, and two ACL-TOP 750s. We still have a water bath, but this is primarily used to defrost frozen samples, and manual clotting tests are only used to teach visiting medical students. We have migrated across several methodologies in the 45-year history of the local laboratory. Laurel gel rockets, used for several assays in the 1980s, were replaced with enzyme-linked immunosorbent assay assays and most assays were eventually placed on automated instruments. Radio-isotopic assays, used in the 1980s, were replaced by an alternate safer method or else abandoned. Test numbers have increased markedly over time. The approximately 31,000 hemostasis assays performed at the Westmead-based laboratory in 1983 had become approximately 200,000 in 2022, a sixfold increase. Some 90,000 prothrombin times and activated partial thromboplastic times are now performed at this laboratory per year. Thrombophilia assays were added to the test repertoires over time, as were the tests to measure several anticoagulant drugs, most recently the direct oral anticoagulants. I hope my personal history, reflecting on the changes in hemostasis testing over my career to date in the field, is found to be of interest to the readership, and I hope they forgive any inaccuracies I have introduced in this reflection of the past.

Keywords
- hemostasis
- thrombosis
- laboratory tests
- methods
- instruments
- history

* *Dedicated to the memory of Prof Jerry Koutts (1944-2013), who facilitated my start in this field.*

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There is no certainty in change, other than change is certain. There are no guarantees in transformation, other than transformation is guaranteed.

Given the historical issues being prepared for *Seminars in Thrombosis and Hemostasis* (STH) to celebrate 50 years of publishing, I thought the readership might be interested in my own personal story, how I began my path in the field and how all this intertwines with the journal that I now find myself spearheading. I hope the readership also forgives what some might describe as my vanity project.

My Early Career

My path in science began many decades ago, first as a youngster with an inquisitive mind, perhaps leading me to my graduate degree in science, with eventual majors in physiology and biochemistry, honors in the field of neurophysiology under the direction of Professor William (Liam) Burke, and my first job in a university laboratory studying salivary secretion in mammals under the direction of Professor John Atherton Young. All of this latter formal study was accomplished at my Alma mater, Sydney University. That first paid scientific job was also perhaps just something to whet the appetite, or perhaps to wet the whistle (given the saliva reference), and since it also led to my first publication.[1]

My second paid scientific job took me to the Institute of Clinical Pathology and Medical Research (ICPMR) in 1982, by then based at Westmead Hospital, where I joined a small research team working in malignant hematology. I stayed with the "Leukaemia Cell Biology Research Unit" for the next 5 years, also acquiring my Ph.D. (again from Sydney University, but from the faculty of Medicine) on a part-time basis. My university supervisor was Professor Peter Castaldi and my local supervisor Dr Kenneth Bradstock who was also the head of the Leukaemia Research Unit. My main output at this facility, both within my employment as a "Research Assistant" and then a "Research Officer," and as part of my PhD, was the production and characterization of a range of monoclonal antibodies against various hemopoietic cells, both normal and malignant cells. We were trying to learn what was different about malignant cells so that we could target and destroy these cells, without damaging normal cells, and thus "cure" leukemia. We were quite successful in the production of these monoclonal antibodies, although most eventually found a better use for flow cytometry-based cellular characterization studies than for leukemia therapy per se. My colleagues and I produced a large series of monoclonal antibodies that we prefixed "WM" (for Westmead) or "AK" (for the lead scientist Arnold Kabral). Many of these antibodies were eventually commercialized by the organization, and several are still commercially available and used (primarily in flow cytometry) by thousands of laboratories around the world. We published our initial research findings in many well-respected peer reviewed hematology journals, including the British Journal of Haematology, the American Journal of Hematology, and Leukemia Research.[2–12] The monoclonal antibodies included WM-15 and WM-47 (against Cluster of Differentiation [CD]-33); WM-53 and WM-54 (CD-33); WM-25 and WM-35 (CD-1); WM-21 (CD-10); WM-12 (CD-11); WM-59 (CD-31); WM-23, AK-1, AK-2, AK-3 (CD-42b); AK-7 (CD-49b); AK-4; and AK-6 (CD-62P), just to name a few of the more successful clones. I do not follow the field too closely anymore, but I understand that great strides are now being made in leukemia therapy, particularly with novel treatments such as CAR-T cell therapy.[13–15]

My Move into the Fields of Hemostasis and Thrombosis

Although successful, most positions held within the Leukaemia Research Unit were grant funded and subject to ongoing success in grant applications. As I had also become a family man during this tenure, with several small children, the decision was made to move to more secure work, and I found myself successful for a job application as a hospital scientist, within the same hematology department, but within the hospital-funded coagulation laboratory in 1987. I was given this position by Prof Jerry Koutts, who was the clinical head of this section at the time. I knew nothing about coagulation at that time, but I had no doubts that I could quickly learn. The position was essentially created as a developmental science-based position, for which I was suitably qualified. The department wanted to develop a range of new tests of hemostasis, or improve methodologies used for existing tests, to improve the diagnosis of hemostasis disease, and I convinced Prof Koutts, and his colleague Dr Thomas Exner, the then lead scientist of the coagulation section, that the easiest path forward was to move the skills I developed within the Leukaemia Research Unit to the coagulation laboratory, and so the Westmead Hospital "Haemostasis and Thrombosis Research Unit" was essentially formed. Prof Koutts was also the head of the Clinical Haematology Department within Westmead Hospital, and indeed, the ICPMR was also part of Westmead Hospital at the time. The first few projects we started included an expansion in the repertoire of monoclonal antibodies and their further characterization into the hemostasis field, beginning with endothelial cells,[16–18] and marking my first publication in a Thrombosis and Hemostasis Journal.[17]

I had also honed my skills with the emerging technology called enzyme-linked immunosorbent assay (ELISA) while part of the Leukaemia Research Unit, and so one of my initial "developmental" tasks was to set up an ELISA for von Willebrand factor (VWF) collagen binding (VWF:CB), which had only recently been described by the pair of Brown and Bosak.[19] Although perhaps contrary to "popular belief," I did not "discover" the VWF:CB, but I did eventually find more value in the assay than Brown and Bosak had reported; the pair had essentially concluded that the VWF:CB ELISA represented "a possible replacement assay for the laborious and imprecise VIII:RCO [VWF:RCo] method of measurement of *in vitro* VWF functional activity."[19] In hindsight, I have previously reflected that the original ELISA method reported by Brown and Bosak[19] was not properly optimized to detection

of high molecular weight VWF or even discrimination of type 1 vs 2 von Willebrand disease (VWD).[20] We published our first report on the VWF:CB in 1991.[21] VWD and VWF were eventually destined to become two of my favorite topics, and PubMed now identifies I have published over 180 papers related to VWD and VWF since my first "seminal" paper on the VWF:CB.[21]

My early time in the "Hemostasis and Thrombosis Research Unit," or essentially the Westmead Coagulation laboratory, were very productive for the unit and nicely complimented the work my colleague Dr Thomas Exner was undertaking, given he was primarily interested in laboratory testing for lupus anticoagulant (LA).[22–26] We found ourselves splitting up the diagnostic assays. Dr Exner focused on LA testing, at that time manually performed using the Kaolin Clotting Time (KCT), introduced the anticardiolipin (aCL) antibody assay and also introduced multimer assays for VWF. I focused on basic VWF tests and VWD and the development of new assays or improving methodology. The remaining staff were mostly assigned to performance of the other assays, including routine coagulation, skin bleeding times, antithrombin III (ATIII), and factor assays. However, it is important to note that I was also involved in physically performing these routine and diagnostic assays as well. We also took turns to perform manual prothrombin times (PTs) for the weekly warfarin clinic. I became quite proficient at manual clot-based assay testing!

Evolution of Hemostasis Testing—The Early Years (the 1980's)

My early work in the hemostasis laboratory at Westmead reflected a balance between research/development activities and routine "hemostasis diagnostics." Thus, in addition to my assay development duties, I was also responsible for overseeing the day-to-day performance of routine coagulation and diagnostic hemostasis tests, given the ICPMR performed pathology testing for Westmead Hospital. We also provided testing for an international normalized ratio (INR) clinic for warfarin patients. Some of the hemostasis testing was performed using manual clot-based tests, using test tubes, pipettes, stop watches, and a water bath. The department had, however, already made a foray into automation, having recently purchased a Coag-A-Mate X2 instrument from a company then called "General Diagnostics"; still, the instrument utilized a series of tubings that had to be rinsed and changed/redirected to enable a change of coagulation assay menu, or to perform factor assays, and so the instrument was mostly just used as a workhorse to perform routine PTs and activated partial thromboplastin times (APTTs). "Urgent" factor assays were performed using manual clot-based methods, employing a metal clip to enable clot detection in up to four tubes at a time. Lupus assays, then primarily using KCT, were also similarly performed manually.

Of course, these were not the only tests we performed. As a "special test" laboratory, we felt we performed a "full repertoire" of hemostasis tests, as well as other tests favored at that time, and planned to develop more. A list of tests the laboratory performed in my first year in the laboratory (1987) is given in ►**Table 1**. The laboratory had just migrated away from performing fibrin/ogen degradation products (FDPs) to D-dimers using a newly developed semiquantitative latex agglutination method and was also using 96 well microtiter plates and plate readers to capture end-point readings for chromogenic "activity" assays (protein C and ATIII) or ELISA (VWF antigen [VWF:Ag] and protein C) but was also still using Laurel gel rocket electrophoresis methodology[27] for some assays (protein S; previously also for protein C and VWF:Ag), probably because of a lack of availability of commercially tagged antibodies to enable ELISA testing. All these "specialized" assays were performed using what we called "in house" methods (or what U.S. laboratories call laboratory-developed tests; LDTs). We used commercial supplies of most test components and matched these reagents to an assay that we developed in house. We were also using some radioisotopic procedures. These were used for the detection of factor VIII antigen and for VWF multimers.

Some test numbers for those early years are shown in ►**Fig. 1**, and a summary of some highlights from this period is provided in ►**Table 2**.

Evolution of Hemostasis Testing—the 1990's

By 1990, the laboratory had already doubled the total number of tests it performed (approximately 70k) compared with 1983 numbers (approximately 35k; ►**Fig. 1**). The number of factor assays had quadrupled from approximately 200/y in 1983 to approximately 800/y in 1990. This period also saw the laboratory establish the VWF:CB for use in VWD diagnostics in 1990, as well as establishing several "thrombophilia"-based assays (in particular protein C and S), as well as progressing several changes in methodology. We had completely phased out our Laurel gel Rockets by 1990, replacing the last such assay (for protein S) by ELISA. The laboratory had already set up an ELISA assay for aCL antibodies in 1986, and we added the Russell Viper venom time assay to the KCT assay for LA testing.

We thought we were a busy laboratory and were having a difficult time keeping up with our solitary Coag-A-Mate X2, so we successfully applied for special purpose funding to purchase an ACL-300R from Instrumentation Laboratory in the early 1990's. We continued to outgrow our automated instrument lines and acquired an MDA-180 from Organon Teknika in the late 1990's.[28] And yet, the test numbers continued to grow, in part because Westmead Hospital also grew, adding more bed numbers every year, and in part because we expanded our reach from being a provider of services to just Westmead Hospital, to a range of additional referral sites.

The 1990's also saw us splitting the hemostasis laboratory and moving the routine assays into a purpose built "core" laboratory, which also housed routine hematology and chemistry tests. The routine coagulation tests (PT, APTT, D-dimer, fibrinogen, and thrombin time) then became available 24 hours a day, 7 days a week ("24/7"). The remaining assays became part of what we called the Diagnostic

Table 1 Test list for the hemostasis laboratory in my first year of service (1987)

Test	Abbreviation	Notes
Prothrombin time	PT	Coag-a-Mate X2
Activated partial thromboplastin time	APTT	
Thrombin time	TT	Manual clot (tubes, pipettes, stop watches, water bath); batch factor assays sometimes performed on Coag-a-Mate X2
Reptilase time	RT	
Echis time	ET	
Fibrinogen	Fgn	
Factor assays (II, V, VII, VIII, IX, X, XI, XII)	FII, FV, FVII, FVIII, FIX, FX, FXI, FXII	
Lupus anticoagulant (by kaolin clotting time)	LA	
D-dimers	DD	Semi-quantitative latex agglutination; had just replaced Fibrin/ogen degradation products
Skin bleeding time	SBT	Template device
Factor XIII	FXIII	Clot solubility
Factor VIII antigen	FVIII:CAG	Radio immunoassay
von Willebrand factor antigen	VWF:Ag	ELISA (previously Laurel gel rockets)
VWF ristocetin cofactor	VWF:RCo	Platelet aggregometry
VWF multimers	VWF:Mult	Radio gel electrophoresis
Antithrombin III (activity)	ATIII	In house chromogenic assays using 96 well plates
Protein C (activity)	PC:Act	
Protein C (antigen)	PC:Ag	ELISA
Protein S (antigen)	PS:Ag	Laurel gel rocket for total protein S; ELISA established in following year
Anticardiolipin antibody	aCL	ELISA
Platelet function	PltFun:Agg	aggregometry
Platelet antibodies	PltAb	Immunofluorescence or aggregometry depending on type
Heparin assay		In house assay
Plasminogen		In house assay
Snake venom identification		Kit from CSL
Fibronectin		In house ELISA
Alpha 2 antiplasmin		In house assay

Abbreviations: CSL, Commonwealth serum laboratories; ELISA, enzyme linked immunosorbent assay; VWF, von Willebrand factor.

Haemostasis Laboratory (DHL), with the apt motto of "we deliver." I was still involved in all aspects of hemostasis, since we still managed the "science" of hemostasis testing, including assessment of routine reagents, although the implementation or performance of the tests themselves were now devolved to 24/7 technicians. Assessment of routine reagents included heparin and factor sensitivity for the APTT, derivation of a heparin therapeutic range for the APTT, and verification/calculation of international sensitivity index (ISI), and mean normal prothrombin time (MNPT) for INR calculations.

Another major highlight of this period was our acquisition, in 1997, of the newly described platelet function analyzer (PFA)-100,[29,30] again from another special purpose grant. This was to prove a valuable acquisition and tied in well to our developmental work on VWF/VWD and the VWF:CB. That our laboratory was one of the first few hundred worldwide to have acquired a PFA-100 that is probably identified by the instrument's serial number (275). We published our first paper on the PFA-100 in the American Journal of Hematology in 1999.[31]

As part of a separate project, primarily undertaken by a PhD student named David Facey, I was also involved once again in the production and characterization of a range of monoclonal antibodies, this time against VWF. We produced several such antibodies and used these to help characterize VWF function.[31,32]

Other changes included the introduction into the laboratory of a new test called activated protein C resistance, which was making quite a stir in the literature as a major cause of thrombosis. Skin bleeding time tests, once very popular, were phased out in 1993.

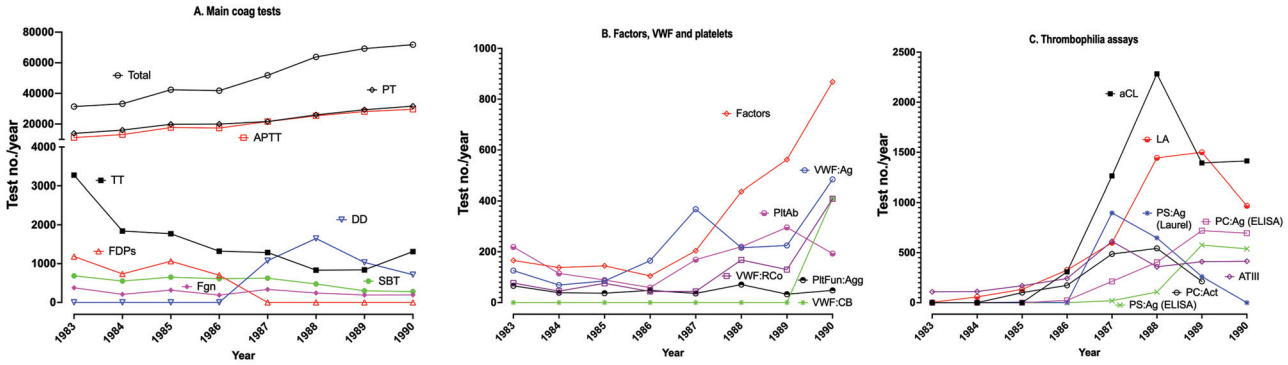

Fig. 1 Test number changes in the early years (1983–1990) of the Westmead coagulation laboratory. (**A**) The main coagulation assays (APTT, activated partial thromboplastin time; D-D, D-dimer; FDPs, fibrin/ogen degradation products; Fgn, fibrinogen; PT, prothrombin time; SBT, skin bleeding time; TT, thrombin time). Total test counts, PTs and APTTs doubled over the period 1983 to 1990. SBTs decreased over time as we introduced a restrictive policy and were eventually abandoned in 1993. FDPs were phased out in 1997 and replaced by D-dimers. (**B**) Factors, von Willebrand factor (VWF) assays, and platelet (plt) procedures (PltAb, platelet antibodies; PltAgg, platelet function by aggregometry). Factor assays quadrupled over the period, largely driven by factor VIII (FVIII) assays. The VWF collagen-binding (VWF: CB) assay was introduced in 1990. PltAbs were performed mostly in thrombocytopenic patients to assess for autoimmune events, on occasion related to drug use (e.g., quinine, heparin). (**C**) Thrombophilia assays. The anticardiolipin antibody (aCL) assay was introduced (as an in-house ELISA) in 1986 because Dr Exner had an interest in antiphospholipid antibodies (aPL), especially the lupus anticoagulant (LA). Antithrombin (AT) was then called Antithrombin III (ATIII). Protein C (PC) was primarily performed by ELISA as an antigenic assay. Protein S (PS) was introduced in 1987 and initially performed by Laurel gel rockets and later performed by ELISA and was for "total" PS. LA comprised the kaolin clotting time (KCT) by manual clotting assay. ELISA, enzyme-linked immunosorbent assay.

Table 2 A summary of highlights in the evolution of hemostasis—a personal perspective from Westmead

Period	The highlights
1980s	A period of considerable change Start of decade primarily focused on establishing the coagulation laboratory to provide a pathology service to the recently build Westmead Hospital Mostly manual coagulation tests in the early 1980s Mostly in house (laboratory developed) tests for the "special hemostasis" assays The laboratory acquired its first "automated" coagulation instrument, the Coag-A-Mate X2 in the mid-1980s Fibrin/ogen degradation products changed to use of D-dimer assays Antigenic assays performed as Laurel gel rockets in the early 1980s migrated to ELISA We were using some radioisotopic assays (FVIII:CAG, VWF multimers) The laboratory doubled the total number of tests performed from the start of the decade to decades end
1990s	A period of considerable change We acquired an ACL-300R in the early 1990's and an MDA-180 in the late 1990's In house VWF:CB ELISA assay developed at the start of the 1990's to supplement VWF:Ag (ELISA) and VWF: RCo (platelet agglutination using the aggregometer) We established some "new" thrombophilia assays (in particular protein C and S, and activated protein C resistance) Protein C and S assays were primarily by ELISA ("antigen"; protein S was "total" [not "free"]) Use of Laurel gel rockets fully phased out in 1990 Skin bleeding time tests fully phased out in 1993 The Russell Viper venom time assay added to the established kaolin clotting time assay for lupus anticoagulant investigation Laboratory acquired the platelet function analyzer (PFA)-100 in 1997 The coagulation laboratory was split into routine coagulation tests, which were placed into a purpose-built ("Core") laboratory to provide 24/7 testing, alongside routine hematology and chemistry, and a "Diagnostic Haemostasis Laboratory" (DHL), which provided specialized hemostasis testing Mon-Fri, 9 to 5. The laboratory had doubled the total number of tests it performed in 1990 (approximately 70k) compared with 1983 numbers (approximately 35k).
2000s	A period of considerable change The DHL acquired a Dade Behring BCS XP and a refurbished Stago StaR Evolution. VWF:RCo migrated from aggregometry to automated assay on the BCS XP The ACL-300R was decommissioned

Table 2 (*Continued*)

Period	The highlights
	Protein S "free" was introduced Test numbers continued to expand Laboratory became involved in many international initiatives, including the International Society on Thrombosis and Haemostasis Society Scientific Standardisation Committee for VWF/VWD Laboratory assisted in development of many Royal College of Pathologists Quality Assurance Program hemostasis proficiency test surveys
2010s	A period of considerable change Stago awarded contract for supply of hemostasis instruments ($n = 35$) and reagents to our Pathology West Network of 27 laboratories Westmead acquired new Stago StaR instruments (x2), a PFA-200, a CS-5100, and an AcuStar instrument The BCS XP was decommissioned Many new tests added to DHL test repertoire VWF:Ag and VWF:CB migrated from ELISA to AcuStar VWF:RCo migrated from BCS XP to CS-5100 and then to AcuStar Protein C antigen assay replaced by activity assay Introduction of direct oral anticoagulants (dabigatran, rivaroxaban, and apixaban) and drug-specific testing Expansion of anti-Xa testing from unfractionated heparin and low molecular weight heparin to measure extensive range of anti-Xa agents VWF multimers added to test repertoire once again (HydraSys Hydragel semiautomated method)
2020s	A period of ongoing change Coronavirus disease 2019 (COVID-19) becomes a pandemic D-dimer testing increase almost 10-fold during waves of COVID-19 Werfen awarded contract for supply of hemostasis instruments ($n = 75$) and reagents to the entire NSWHP Network of 60 laboratories. Westmead acquires 2x ACL-750s. NSWHP harmonizes all routine coagulation tests to same instrument line (ACL-TOPs) and reagents (Werfen) across all 75 instruments/60 laboratory sites NSWHP harmonizes all specialized hemostasis tests performed on the ACL-TOP platform to use same methodology and reagents (Werfen or otherwise) across all instruments/laboratory sites performing the same tests NSWHP continues to harmonize all other assays performed by different sites (e.g., establishes a common normal reference range for PFA-100/-200 testing) The Stago StaR instruments decommissioned Drug measurement assays moved from DHL to Core laboratory to enable 24/7 availability

Abbreviations: CSL, Commonwealth serum laboratories; ELISA, enzyme-linked immunosorbent assay; NSWHP, NSW Health Pathology; VWD, von Willebrand disease; VWF, von Willebrand factor.

Evolution of Hemostasis Testing—The New Century Cometh (the 2000's)

The laboratory continued the acquisition of automated instrumentation in the 2000's, acquiring a Dade Behring BCS XP in the last half of the decade for the specialized hemostasis laboratory (DHL). The Westmead site also acquired a refurbished StaR Evolution from Diagnostica Stago in 2009. The ACL-300R was eventually decommissioned. The protein S assay previously performed as a "total" protein S assay was supplemented with a "free" protein S assay, still by ELISA. Test numbers continued to grow.

The VWF:RCo previously performed by platelet aggregometry was migrated to the BCS XP, becoming automated. We also introduced additional modifications to improve sensitivity to low levels of VWF.[33]

We were very prolific in publishing during this period and were involved in many research projects. Many of the publications were related to VWF testing and VWD. I helped set up a range of hemostasis proficiency test surveys for the Royal College of Pathologists Quality Assurance Program (RCPAQAP). I was active in the International Society on Thrombosis and Haemostasis Society Scientific Standardisation Committee for VWF/VWD, and we published the update on the pathophysiology and classification of VWD headed by J Evan Sadler.[34] Our laboratory became the lead test site for a multicenter study to compare pharmacokinetics of two factor concentrates (Biostate and AHF [High Purity] in people with VWD,[35] which formed the basis of the regulatory approval of Biostate in Australia, a plasma-based VWF/FVIII concentrate still used today as a therapy for VWD.

Evolution of Hemostasis Testing—the 2010's

This period was another of great change for the laboratory. NSW Health Pathology (NSWHP) was formed in 2012. The ICPMR initially became part of the "Pathology West" group of (27) laboratories. At the start of the decade, we were also charged with progressing a public tender for standardization of hemostasis instruments across all 27 "Pathology West" laboratories. The Diagnostica Stago group were named the successful supplier in 2012, and 35 instruments were eventually deployed to the 27 laboratories over the subsequent years. The ICPMR acquired two StaR Evolution instruments,

one for the Core laboratory for routine coagulation testing 24/7 and the other for the specialized hemostasis laboratory (DHL), in 2013. We also acquired a CS-5100 at this time to supplement hemostasis testing not available on the Stago platform. The BCS XP was sadly decommissioned in 2015. VWF:RCo assays were migrated across from the BCS to the CS-5100, but we managed to maintain the low-level VWF sensitivity previously established.[33] We replaced the protein C antigen assay with an activity (chromogenic) assay.

The specialized laboratory also acquired a new PFA-200 in 2013 to supplement the PFA-100, which by this stage was over 15 years old, and we felt potentially set to fail us. Despite the advanced user interface, we had several problems with the initially installed PFA-200, eventually and sequentially receiving two replacement instruments during the first 2 years of placement, and forcing us to continue to rely on our "aging" PFA-100.

This decade also saw us acquire the ACL AcuStar, which we initially installed to assess the manufacturer's newly developed VWF:CB assay.[36] I saw no logic in just evaluating the new VWF:CB assay as a potential replacement to the VWF:CB ELISA assay, and thus, we evaluated the three VWF test panels on the AcuStar against the three current VWF tests as reference.[36] We used the data to enable the move of all three VWF tests to the AcuStar platform, retaining the existing assays (VWF:Ag and VWF:CB by ELISA; VWF:RCo on CS-5100) for research purposes and for special patient investigations. We also then used the AcuStar to assess, and eventually adopt, a range of other useful hemostasis assays. First, we started performing assays for heparin-induced thrombocytopenia (HIT) again. Eventually, we also migrated the aCL from ELISA to the AcuStar and also introduced the anti-β-2-glycoprotein I (aβ2GPI) assay using the AcuStar.

This decade also saw the introduction of a range of direct oral anticoagulant (DOAC) drugs, marketed by the drug companies as not needing to be monitored (and thereby aiming to capture the profitable warfarin for atrial fibrillation market), but which we were being increasingly asked to measure. So, we set up a range of drug measurement assays, including for dabigatran, rivaroxaban, and apixaban.

The last half of the decade saw us reintroducing VWF multimer testing after a hiatus of several decades at Westmead. This was achieved using a new semiautomated methodology using a Sebia HydraSys instrument.[37]

As the decade drew to a close, we began to hear of a new viral infection with origin in Wuhan, China. This was later identified to be a new strain of severe acute respiratory syndrome coronavirus (SARS-CoV-2). Little did we know what was to come.

Evolution of Hemostasis Testing—The Contemporary 2020's

It goes without saying that the most significant event of 2020 was the identification of COVID-19 (coronavirus 2019), caused by SARS-CoV-2, as a pandemic. Drug companies scrambled to develop drugs and vaccines to combat COVID-19, and remarkably, the first few vaccines were developed and approved for use before the end of 2020. The timeline for the contemporary 2020s is short. At time of publication, we have just started into 2023. And yet, much has happened in the hemostasis field in these 3 long years. At time of writing, a PubMed search of COVID-19 identified over 325,000 citations, with similar numbers (respectively, approximately 94,000, approximately 140,000, approximately 125,000) in each year (2020, 2021, 2022). Most notable for our field is that although SARS-CoV-2 infection primarily attacks the lungs, COVID-19 itself is largely reflective of a prothrombotic disorder, often driven by a condition termed COVID-19-associated coagulopathy, and reflecting eventual thrombosis (or microthrombosis), and leading to multiorgan damage.[38]

Within our own laboratory, we identified a surge is test numbers for some tests, but mostly for D-dimer, which is both a marker of thrombosis and also a prognostic marker for COVID-19-associated adverse outcomes.[39,40] Indeed, the entire network of 60 laboratories within NSWHP reported a huge increase in D-dimer requests that could be linked to various waves of SARS-CoV-2 infections and, in some sites, reflecting nearly 10x increases in test requests over similar periods prior to COVID-19.[41]

In terms of automation, the entire NSWHP group of (60) laboratories were part of a NSW state-wide tender for instrumentation, which was awarded to Werfen, and the ACL-TOP 50 group of analyzers (350, 550, 750) were rolled out to 60 laboratories (for a total of 75 instruments). Our two Stago StaR Evolutions were replaced with two ACL-TOP 750 instruments and the Stago instruments decommissioned. Our Westmead laboratory was at the forefront of the statewide instrumentation and test methodology verification process[41–45] and also led several other NSWHP-harmonization initiatives, including for the PFA-100/-200.[46]

►**Table 3** provides a list of hemostasis tests currently performed by the Westmead laboratories.

Health Infrastructural Changes—Some Additional Contextual Background

For added context, it may also be useful for the reader to be taken through a journey of the local health structures throughout the past 40 years. The ICPMR was moved from the suburb of Lidcombe to Westmead in 1977 to become the pathology service provider to the newly constructed Westmead Hospital, destined to become the largest public hospital in Australia and to service the growing population of the western suburbs of Sydney, then representing the highest population growth base of the entire state of New South Wales (NSW). At the beginning, the ICPMR and Westmead Hospital were a combined unit (►**Fig. 2**). Soon after construction, Westmead Hospital became a part of the Parramatta and Cumberland Hospital group, but this did not change the relationship with the ICPMR, which remained part of Westmead Hospital. The structure of NSW health changed in 1986, when 17 Area Health Services were created, and Westmead Hospital became part of the Western Sydney Area Health Service (WSAHS).[47] Still, the ICPMR remained a part of Westmead Hospital, and thus part of this newly

Table 3 Test list for the Westmead hemostasis laboratories in 2023

Test	Abbreviation	Notes
Prothrombin time	PT	ACL-TOP 750; Werfen reagents; tests performed in Core laboratory and available 24/7
Activated partial Thromboplastin time	APTT	
Thrombin time	TT	
Fibrinogen	Fgn	
D-dimers	DD	
Unfractionated heparin anti-Xa	Anti-Xa (UH)	ACL-TOP 750; primarily Werfen reagents (some assays may use additional non-Werfen reagents/calibrators/controls); tests performed in Core laboratory and available 24/7
Low molecular weight heparin	Anti-Xa (LMWH)	
Danaparoid (Orgaran)	Anti-Xa (Dan)	
Fondaparinux	Anti-Xa (Fon)	
Apixaban	Anti-Xa (Apix)	
Rivaroxaban	Anti-Xa (Riv)	
Dabigatran	Dilute TT	
Factor assays (II, V, VII, VIII, IX, X, XI, XII)	FII, FV, FVII, FVIII, FIX, FX, FXI, FXII	ACL-TOP 750; Werfen reagents; tests performed by DHL
Protein C (activity)	PC:Act	
Protein S - free	PS:free	
Antithrombin (activity)	AT	ACL-TOP 750; non-Werfen reagents; tests performed by DHL
Lupus anticoagulant (by APTT and Russell Viper Venom Time)	LA	ACL-TOP 750; Werfen RVVT reagents and Cephen APTT reagents; tests performed by DHL
Activated protein C resistance	APCR	ACL-TOP 750; Pefakit tests; performed by DHL
von Willebrand factor antigen	VWF:Ag	AcuStar; Werfen reagents; tests performed by DHL
VWF ristocetin cofactor	VWF:RCo	
VWF collagen binding	VWF:CB	
anticardiolipin antibody (IgG and IgM)	aCL	
Anti-β-2-glycoprotein I (IgG)	aβ2GPI	
ADAMTS-13	ADAMTS-13	
Heparin-induced thrombocytopenia	HIT anti-PF4/H	
VWF multimers	VWF:Mult	Sebia Hydrasys method; performed by DHL
VWF:FVIII binding assay	VWF:FVIIIB	Stago ELISA; performed by DHL
Platelet function—aggregometry	PltFun:Agg	AggRAM aggregometer; performed by DHL
Platelet function—PFA-100/-200	PFA	PFA-100/-200; performed by DHL

Abbreviations: DHL, Diagnostic Haemostasis Laboratory (specialized coagulation test laboratory)—operates M to F, 8 am to 5 pm; ELISA, enzyme linked immunosorbent assay; PFA, platelet function analyser; VWF, von Willebrand factor.

formed WSAHS. In 2004, the health structure changed again and the number of Area Health Services were reduced from 17 to 8. The WSAHS and the Wentworth Area Health Service were combined to form a larger unit called the Sydney West Area Health Service (SWAHS). Again, the ICPMR remained part of Westmead Hospital and thus SWAHS. SWAHS was abolished on 1 January 2011 by the Health Services Amendment (Local Health Networks) Act 2010 (Act No.97, 2010), when it was replaced by the Western Sydney Local Health Network and the Nepean Blue Mountains Local Health Network. At this time, the ICPMR started its move away from the hospital networks/districts to become part of autonomous pathology groups.

NSWHP was formed in 2012. At the beginning of this process, the ICPMR became a part of a 27-laboratory "functional network" called Pathology West. Pathology West was eventually split into two, with metropolitan laboratories forming the "West" network, and rural/regional sites forming the "Rural and Regional network." Still, the same 27 laboratories generally behaved as a single functional unit regarding test harmonization, in particular for hemostasis, and for example all 27 laboratories acquired Stago Instrumentation as a result of a Pathology West tender and all 27 laboratories were on the same (CERNER) laboratory information system. The latest harmonization process occurred in the 2020's and has involved all 60 laboratories in NSWHP harmonizing to

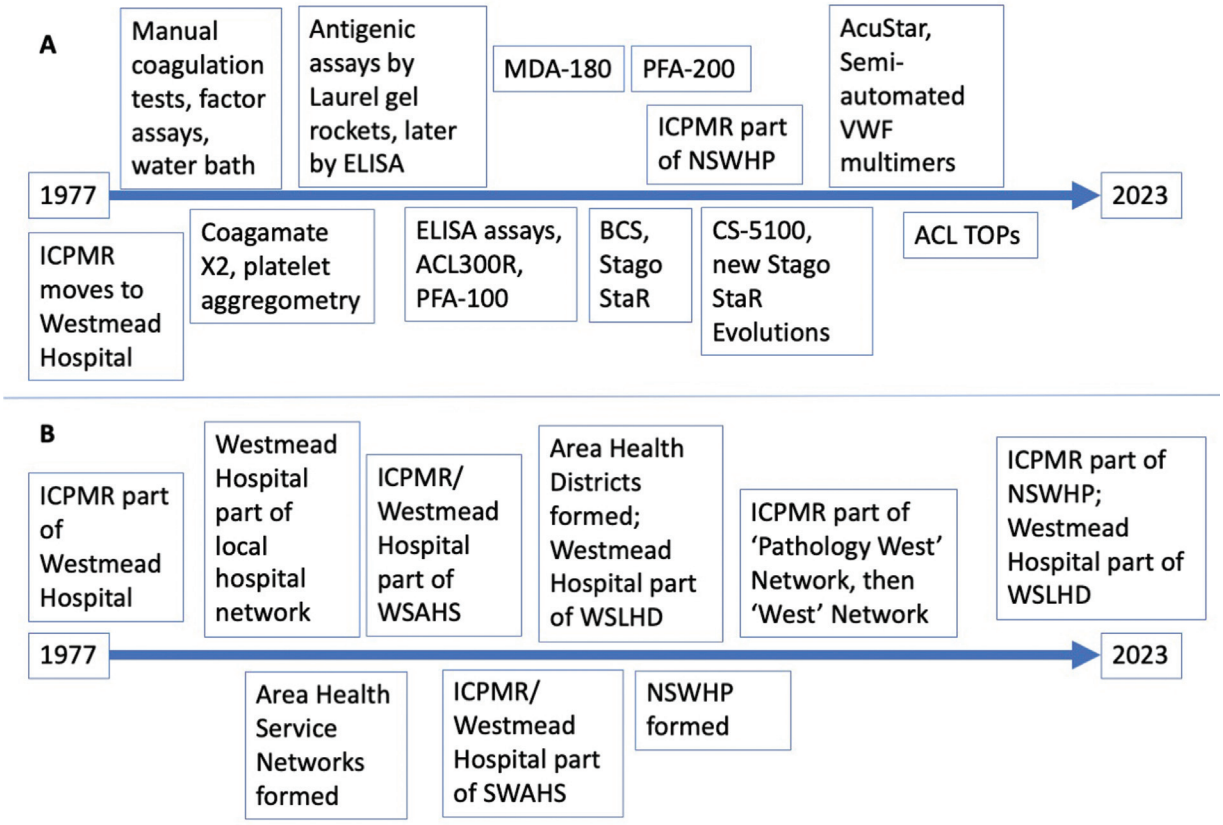

Fig. 2 Summary of some key changes in instruments and methodologies at Westmead, and local health structures 1977–2023, a 45-year history. (**A**) Key changes in instruments and methodologies at Westmead. The Institute of Clinical Pathology and Medical Research (ICPMR) moved from the suburb of Lidcombe to the suburb of Westmead to provide pathology services to the then newly built Westmead Hospital. The coagulation laboratory began offering routine coagulation tests such as the prothrombin time (PT) and activated partial thromboplastin time (APTT), with these initially performed manually with test tubes, pipettes, stopwatches, and a water bath. The laboratory then acquired a Coag-A-Mate X2 to become a work-horse to perform the majority of PT and APTT assays, although manual clot assays were still performed for urgent tests and for the warfarin clinic. Antigenic assays were initially performed by the Laurel gel rocket procedure, and then by ELISA, and eventually most tests moved to automated analyzers. The laboratory then acquired an ACL-300R, a PFA-100 and an MDA-180, and later a BCS, StaR, a CS-5100, and PFA-200. An AcuStar and the ACL-TOP analyzers followed. (**B**) Key changes in the health structure. The ICPMR moved to Westmead Hospital to provide its pathology services and was initially part of Westmead Hospital. Westmead Hospital then became part of a small consortium of local health services called the Parramatta/Cumberland hospitals. Area Health Service "networks" were then formed and the ICPMR and Westmead Hospital became part of the Western Sydney Area Health Service (WSAHS), which was later merged with another AHS, and the ICPMR and Westmead Hospital became part of a larger Sydney West Area Health Service (SWAHS). NSW Health Pathology (NSWHP) was formed in 2012, and the ICPMR became part of a 27-laboratory network called "Pathology West." At the same time, SWAHS was disbanded and local health districts were formed, with Westmead Hospital becoming part of the Western Sydney Local Health District (WSLHD). Pathology West was split into metropolitan sites (West) and 'Rural/Regional' sites. As of 2023, the ICPMR is part of NSWHP and thus separate to Westmead Hospital, which is part of the WSLHD. However, the ICPMR maintains its presence on the same site it moved into in 1977 and, thus, physically part of Westmead Hospital.

the same instrument platforms and tests; for hemostasis, this has involved the roll-out of 75 ACL-TOP instruments to 60 NSWHP laboratories, and harmonization of all tests performed on that platform across all sites.[42] Specialized assays also performed on the ACL TOP platform in a smaller number of NSWHP sites have also been harmonized across all sites performing the same assays.[43–45] Additional hemostasis tests not performed on the ACL-TOP platform (e.g., PFA-100/-200 tests, AcuStar tests) are going through a separate harmonization process. For example, we have harmonized normal ranges for PFA-100/-200 to single harmonized ranges across all sites with PFA-100/-200 instruments.[46] Further harmonization with NSWHP will ultimately be facilitated by a common laboratory information system and a common quality system (both strategies are being progressed; this project is called "Fusion").[48] The successful tender for the laboratory information system has just been announced. Harmonization is being facilitated by the construction of a common NSWHP test catalogue.[49]

The main point of mentioning all this "health structure" transformation is to highlight that there has also been much change in the public NSW Health system structure over the past 40 years; all of this chronological change has had a significant impact on pathology service delivery and our interaction with what have now become our health "partners" (i.e., the public hospital system). These changes have taken place in the background, outside the control of the laboratories providing pathology testing, but these significant changes had to be managed while maintaining our pathology service. The ICPMR was once a part of Westmead Hospital, even throughout the various iterations of the Parramatta/Cumberland Hospitals group, the WSAHS, and

the SWAHS, but this changed when the LHDs and NSWHP were created; the ICPMR is now part of NSWHP, albeit still occupying the same physical space on the Westmead Hospital Campus as it did when it first started operations at Westmead.

Some Personal Highlights during my 40 Years at Westmead

I have already mentioned smatterings of these personal highlights in the above chronology. As mentioned, it took me nearly 10 years from the end of my BSc degree to my start in the hemostasis field, and I am forever grateful to my mentors and colleagues who helped shape this path. The main legacy of my prehemostasis work is the development and characterization of a large number of hemopoietic/leukemia-related monoclonal antibodies, of which many are now commercially available and assisting thousands of laboratories worldwide in cellular characterization studies for leukemia typing and for research. If the monoclonal antibody is named with a WM- or AK- prefix, it was probably developed by us at Westmead Hospital.

The von Willebrand Factor Collagen Binding (VWF: CB) Assay

As mentioned, I did not "invent" the VWF:CB, but I have certainly done much to facilitate its use in VWD diagnostics. Beginning from our initial studies in the very late 1980's, the VWF:CB was officially incorporated into our test repertoire in 1990 (►**Fig. 1**), to supplement our only alternative functional VWF assay, the classic VWF:RCo assay, then performed by platelet aggregometry. At the beginning, we had envisioned replacing the problematic and time-consuming VWF:RCo with our VWF:CB, but instead soon learnt that the assays were complementary and were best used in parallel. We also identified through continued studies that the VWF:CB, as initially described by Brown and Bosak, was suboptimal for the purpose. It is now over 30 years since we published our first paper on the VWF:CB,[21] and I have published several reviews on the assay,[50,51] although an update is certainly due. Those who have remaining doubts on the utility of the VWF:CB are most probably using, or getting test results from, suboptimal VWF:CB assays; indeed, the most popular VWF:CB worldwide is an ELISA from Technoclone, which we have shown many times to be a suboptimal assay.[52–54] We ultimately abandoned our in-house VWF:CB assay after acquiring the AcuStar, which is the only instrument capable of performing the three test panel of VWF:Ag, VWF:CB and VWF:RCo (as a glycoprotein I recombinant assay, or VWF:GPIbR). This is not to say that there was anything really wrong with our in-house VWF:CB assay, which had served us well over the preceding 25 years. There are also notable differences in the utility of the AcuStar vs in-house ELISA VWF:CB, especially as related to type 2M VWD,[36,55] but we can accommodate this difference in our diagnostic algorithmic strategy. Moreover, it becomes increasingly difficult to travel the path of laboratory accreditation for in-house assays (what the US term LTDs) when a commercial alternative is available; and among the commercial alternatives, the AcuStar method is the definitive pick.[54] As an alternative, for a commercially available ELISA assay, the Stago VWF:CB would be our recommendation.[52]

The notable highlights for the VWF:CB are its supplementary utility as part of a four-test panel for VWD diagnostics (VWF:Ag, VWF:RCo [or a glycoprotein Ib binding alternative], VWF:CB, and factor VIII coagulant [FVIII:C]), and as supported by the United Kingdom Haemophilia Centre Doctors Organization guideline, as approved by the British Committee for Standards in Haematology,[56] ahead of a standard three test panel, as currently proposed by the latest VWD diagnosis guidelines.[57] Thus, a four-test panel is much less likely to be associated with diagnostic errors than a three-test panel.[53,54] This is a consistent finding in our geographic locality, where near equal numbers of laboratories perform a four- versus three-test panel. The VWF:CB also has value in terms of assessing the clinical benefit of a VWD desmopressin (DDAVP) trial, as well as assisting in DDAVP-aided VWD diagnostics where a diagnosis is unclear.[58–60]

There is no doubt that our initial positive experience with the VWF:CB led to my special interest in the field of VWD diagnostics and contributed greatly to the 180-plus papers our laboratory has since published on VWF/VWD from the humble beginning in 1991.[21]

The Platelet Function Analyser-100 and -200

Our laboratory was also one of the first world-wide to utilize the PFA-100, first reported in 1995 in this journal[29,30] and later in 1998.[61] Since our own first publication on the topic in 1999,[31] our laboratory has since published more than 30 papers on the PFA-100/-200. The initial publications focused on VWD identification/exclusion, especially relevant since the PFA-100/-200 can give test results (called a closure time; CT) in $<$15 minutes, whereas VWF tests took in the matter of hours or days to perform. It is not that the PFA-100 can diagnose VWD; it cannot. However, a normal PFA-100 test result can be a good predictor of the absence of significant VWD; indeed, in our last analysis, a normal PFA-100/-200 test finding better identified the absence of VWD than did any single VWF assay.[62] In addition, along with the VWFCB, the PFA is useful in the assessment of VWD DDAVP trials and provides a functional surrogate for improved VWF activity in particular VWD categories, as well as providing potential diagnostic utility.[59,60] Thus, normalization of PFA-100/-200 CTs will typically occur when DDAVP is utilized in type 1 VWD but rarely normalize in type 2A/2M VWD.

Our more recent exploration into the PFA-100/-200 has been in relation to quality control, being the source laboratory for external quality assessment (EQA) challenges sent through the RCPAQAP[63–68] and more recently also External quality Control of diagnostic Assays and Tests.

Semiautomated von Willebrand Factor Multimers

Our laboratory was performing VWF multimers from 1986, before I started in the hemostasis laboratory in 1987; these were performed by my colleague Thomas Exner of LA fame. The procedure involved radioisotopic work. Our laboratory

stopped performing VWF multimers in 1993, when Dr Exner left Westmead to pursue a life in private industry. We did not really miss performing the test; we had a growing aversion to use of radioisotopic procedures in the laboratory, and the assay was very complex and time consuming to perform, requiring many days of work to process some 15 samples. To accommodate the loss of VWF multimer testing, we also developed a test algorithm that did not require the use of VWF multimer analysis and instead incorporating the VWF:CB assay within the diagnostic pathway. That would have been the end of VWF multimers at Westmead, had it not been for the development of the Sebia HydraSys semiautomated system (Hydragel 5 von Willebrand Multimers). We were approached by the local distributor of Sebia to evaluate the method; we had a keen MSc student interested to progress the study, and so VWF multimers were eventually incorporated back into our test panel in 2017.[37] We have published a few additional papers since then utilizing the methodology.[55,69–71] I still contend that appropriate strategic use of a four test panel including the VWF:CB can avoid the need to perform VWF multimers, but as part of a network of 60 laboratories, there was value in having the assay available in at least one NSWHP site and given our expertise in VWD diagnostics, the Westmead site seemed the logical choice. This method certainly beats the old radio-isotopic assay! The Hydragel 5 system (five lanes) has been supplemented with the Hydragel 11 system (11 lanes) permitting nine patient samples plus two controls (a normal and a type 2A/2B sample) per run, and the assay can be completed in a single day (with approximately 4 hours hands on time).

External Quality Assurance

It would be remiss of me to not more fully highlight the role of the Westmead hemostasis laboratory in EQA activities in the field of hemostasis and thrombosis. It is perhaps not well known that the RCPAQAP had its origins dispersed around various localities around Australia, with sections started in various locations according to local interest. The RCPAQAP Haematology section was therefore based in our Haematology Department at Westmead Hospital in the early 1980's, under the leadership of Dr Wilbur Hughes. At the time, various staff in the hematology department held dual roles, working in both hematology testing/diagnostics and the RCPAQAP Haematology. Indeed, many of the staff starting their working career in hematology at Westmead eventually moved to formal positions within the RCPAQAP Haematology when the RCPA Quality Assurance Programs Pty Ltd, a company independent from but aligned with the Royal College of Pathologists of Australasia, was created in 1988.

Thus, a large portfolio of RCPAQAP EQA programs related to hemostasis were initially developed at Westmead Hospital and were formally migrated to the RCPQAP over time. The first of these was a survey of heparin monitoring in Australasia,[72] which was to lead on to the formal initiation of heparin-based EQA programs at the RCPAQAP, of which some have since been reported.[73–75] Unsurprisingly, VWF testing for VWD also featured in early reports from the RCPAQAP Haematology, then still based at Westmead Hospital, including initial surveys,[76,77] upon which to base the subsequent proficient testing program. Indeed, VWF testing for VWD has been a key feature of several reports from the RCPAQAP Haematology since those beginnings, including a strategy for in house preparation of VWD-mimicking surrogates to supplement use of patient samples for EQA.[78–80] This has in large part been assisted by the fact that nearly 50% of RCPAQAP participant laboratories employ a VWF:CB assay, giving this program a somewhat unique insight into VWD diagnostics that cannot be assessed in localities in which the VWF:CB is not typically employed or, if employed, is typically performed by ELISA, such as North America.[81,82] Australian and New Zealand laboratories enjoy a less restrictive regulatory landscape compared to North America, and thus, they are more easily able to take up newer methodologies, such as automated VWF glycoprotein Ib binding assays (i.e., VWF:GPIbR for recombinant GPIb; VWF:GPIbM for mutant GPIb) or take up use of AcuStar VWF assays including the VWF:CB; thus, the RCPAQAP Haematology will continue to be able to provide a unique insight into developments in VWD diagnostics. In some test sites, notably U.S. laboratories, very few VWF activity assays are regulatory cleared, leaving U.S. laboratories at least a decade behind in VWD diagnostics compared with European and Australian/New Zealand laboratories. Our latest Westmead ICPMR/RCPAQAP collaborative publications on VWF/VWD EQA have recently been published, and these provide unique insights into VWD diagnostics in contemporary times.[54,83] Comparisons of EQA findings from Australasia versus other geographies also makes for interesting reading.[84,85]

Naturally, there are many more examples of EQA collaboration between the RCPAQAP and the Westmead laboratory, including antiphospholipid antibody assays such as aCL and aβ2GPI,[86–90] thrombophilia assays,[91–93] factor inhibitors,[94–99] LAs,[100–104] the DOACs,[105–109] and of course, as previously noted, the PFA-100/-200.[63–68,110]

Back to Basics—The International Normalized Ratio

The PT and APTT reflect the most commonly performed hemostasis assays (►**Figs. 1** and **3**). The PT is often reported as an INR for monitoring patients on therapy with vitamin K antagonists (VKAs) such as warfarin. The INR is a simple calculation, being $INR = (\text{patient PT}/\text{MNPT})^{ISI}$. This calculation is meant to better standardize INR test results compared with PT for VKA monitoring. However, the only well-known "certainty" in this calculation is the patient's PT, which is derived from the coagulation instrument. The ISI may be provided as a generic or instrument specific ISI from the PT reagent manufacturer, but this ISI needs to be locally verified before reagent use.[111] The MNPT is never provided by the PT reagent manufacturer but needs to be estimated by the laboratory. There are various recommended procedures for estimation, or verification, of MNPT or ISI,[111] and yet there remains significant variation in laboratory-derived INRs between test sites, as evidenced, for example, in EQA reports.[112] Manufacturers of PT reagents tend to use the World Health Organization (WHO)-recommended method

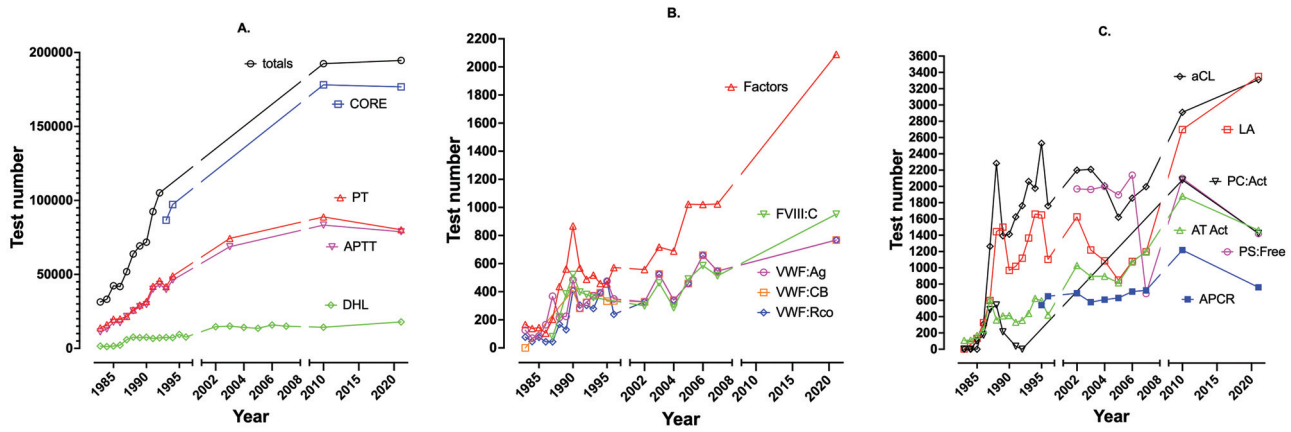

Fig. 3 Test number changes in Westmead coagulation and hemostasis assays over the years 1983–2021. (**A**) Main summary data. Total of all tests performed has increased from approximately 30,000 in 1983 to current levels of approximately 200,000 tests/year, with these mostly comprising tests performed by the core laboratory, which operates 24 hours a day/7 days a week, and primarily for the routine coagulation assays (APTT, activated partial thromboplastin time; PT, prothrombin time; approximately 80,000 tests for each performed for each every year). Specialized hemostasis assays are now performed in the Diagnostic Haemostasis Laboratory (DHL), with nearly 18,000 assays performed in 2021 (compared with approximately 1,500 performed in 1983)—representing a >10-fold increase. (**B**) Factor and von Willebrand factor (VWF) assays. Factors have also increased >10-fold (approximately 170 in 1983 to >2000 in 2021, with around half of these being for FVIII). The laboratory now performs approximately 800 VWF tests per year (using the panel VWF:Ag, VWF:RCo [as VWF:GPIbR], and VWF:CB); this compares with just over 100 tests/year in 1983. (**C**) Thrombophilia assays. The anticardiolipin antibody (aCL) assay, introduced as an in-house ELISA in 1986, is now performed on the AcuStar, as is the anti-β-2-glycoprotein I (aβ2GPI) assay. We performed over 3300 aCL assays in 2021, with similar numbers of lupus anticoagulant (LA) assays. In the past, the LA assay comprised the kaolin clotting time (KCT) by manual clotting assay; this was supplemented with Russell Viper Venom Time (RVVT) testing in the 1990's and later with APTT testing. Activated protein C resistance (APCR) testing was introduced in 1995 and protein S free (PS:Free) in 2002. Protein C (PC) was initially performed as an antigen assay and eventually replaced by the activity assay (PC:Act) in the late 2000's.

for the estimation of ISIs, namely the performance of manual PTs with some at least 20 normal individuals and some 60 samples from stabilized VKA patients using the test PT reagent versus the WHO reference thromboplastin (or a secondary thromboplastin reference traceable to the WHO reference). However, individual laboratories would not be able to perform this onerous procedure to estimate or verify an ISI for their PT reagent and thus use alternatives such as calibration or reference plasma sets.[111] The MNPT can be calculated as the geometric mean of 20 normal individuals.

Our laboratory highlighted the limitations of these approaches in 2008[113] and embarked on a different journey. We showed at that time that the use of different sets of 20 normal individual plasmas would identify different MNPT values and that use of different calibration or reference plasma sets would derive different ISI values. We proposed a simpler approach using linear regression to compare a new PT reagent against the previous PT reagent used as reference. For this process to work, an initial PT reagent would need to be identified as a suitable *reference* PT reagent, and the easiest way to do so was to establish prior good performance in EQA surveys of proficiency. Since that initial report,[113] our laboratory continued to utilize this approach over the subsequent 15 years,[114–116] for example, showing improvement in cross-laboratory performance (by reducing coefficient of variation [CV] values in EQA) using the Pathology West 27-laboratory Network.[116] We have most recently identified the utility of this process in regard to standardizing ISI and MNPT values across the entire NSWHP network of 60 laboratories; thus, using this approach, all sites using a particular lot of PT reagent harmonized to use a single ISI and a single MNPT, for that lot of PT reagent, irrespective of laboratory location and which of the ACL-TOP (350, 550, or 750) instruments are in use.

Heparin-Induced Thrombocytopenia and Vaccine-Induced Thrombotic Thrombocytopenia

The Westmead coagulation laboratory was involved in testing for HIT at the time I started work at Westmead. This was by platelet aggregation, assessing for platelet activation. Once attached to the hemostasis laboratory, I was tasked with developing a local serotonin release assay (SRA) to supplement or potentially replace this aggregometry-based platelet activation/aggregation assay. Although this task proved to be successful,[117] the continued use of radioisotopes, the need for a liquid scintillation counter and for overnight counting, and possibly most importantly the ongoing need to source reactive normal donor platelets (which somehow tended to be mine alone), saw the laboratory eventually give up this procedure at Westmead. A long sojourn in this field followed at Westmead, and we, like many others, outsourced our HIT testing to other more specialized centers. This situation changed after we acquired our AcuStar, which enabled local immunological evaluation of HIT (antiplatelet factor 4/heparin [PF4/H] complex) antibodies.[118] We published our local study on the new chemiluminescence-based assay in 2018,[118] comparing the AcuStar assay against an ELISA, the Stago rapid assay (StiC), and also against SRA, and furthermore subsequently followed up with a multicenter study in 2021.[119] We

determined that the AcuStar assay had an excellent sensitivity and specificity for pathological HIT (or HIT with thrombosis; HITT; as assessed by its surrogate laboratory "gold standard" SRA), especially for high AcuStar values. In the multicenter study, any positive result (value >1.0 U/mL) had a sensitivity of 88.5% (95% confidence interval [CI] 81.8–92.9%), and a high AcuStar result (>10U/mL), a 96.6% specificity (95% CI: 92.2–98.5%), for HITT identified by SRA.

Little did we know then that the COVID-19 pandemic and our attempts to circumvent infections or severe disease using vaccines would lead to a rare event that came to be known as VITT (for vaccine-induced [immune] thrombotic thrombocytopenia), with a pathophysiological basis similar to HITT, with development of anti-PF4 antibodies, albeit this time unrelated to heparin use.[120,121] The COVID-19 pandemic seemed for a time to morph into a "VITT-pandemic," although in reality this adverse event of vaccine use was rare by HIT standards. Whereas HIT can occur in some 1 to 3% of exposures to unfractionated heparin, VITT only occurs in around 1/50,000 to 1/100,000 doses of vaccine, with only adenovirus-based vaccines consistently confirmed as being associated with VITT.[122,123] Although relatively rare, VITT became a major preoccupation of many of us active in the hemostasis field, especially given its connection to COVID-19 vaccine use and thrombosis associated with thrombocytopenia. As part of the THANZ (Thrombosis and Haemostasis Society of Australia and New Zealand) group charged to diagnose and manage the unfolding VITT crisis in Australia, we held regular out of normal work hours Thursday evening meetings for almost a year devising diagnostic and management strategies and discussing our cases.[124] Our most recent publication reflects the largest case number ever evaluated for VITT.[125] Unlike HITT, the anti-PF4 antibodies in VITT are not detected by the AcuStar or by other rapid anti-PF4/H assays, but only by ELISA.[125–129] This may provide a basic strategy for helping to characterize patients as VITT versus HITT, since both exposure to heparin and to vaccination is feasible in a small cohort of patients. Thus, positivity by AcuStar and by ELISA is more likely HIT/HITT than VITT. Moreover, a separate category of anti-PF4 antibody-associated pathology can also occur due to infection, including SARS-CoV-2, or surgery,[120,130–132] thus further complicating differential diagnosis of VITT versus HITT versus infection/surgery-related thrombotic thrombocytopenia, in patients undergoing surgery, or hospitalized with COVID-19, and/or under heparin treatment.

My Connection with Seminars in Thrombosis and Hemostasis

My first publication in STH was a review that I wrote on the VWF:CB in 2002.[50] A few years later, I was invited by Eberhard Mammen, the then editor in chief (EIC) of STH, to guest edit my first issue of STH, this being on the topic of thrombophilia.[133] Although I felt this to be an important topic in hemostasis/thrombosis at that time, my main aim for this issue was to put thrombophilia testing into perspective and to reduce unnecessary thrombophilia testing, since we seemed to be "diagnosing" a lot of protein C and S deficiencies in patients likely to be on VKA therapy (i.e., many false positives).[133] We still have not learnt and are now instead "diagnosing" a lot of LA in patients likely to be on DOAC therapy.[104,134,135] I then guest edited two issues of STH in 2006, these being on my favorite topic of VWD.[136,137] I was made a regional editor of STH in 2006 by Eberhard and tasked to look after the Asia-Pacific region. This restructure was made to help him reduce his foot-print in the journal and to progress the journal with more independence of the EIC, as well as to facilitate the move from six issues a year to eight issues a year. I continued to support STH with contributions and guest editing duties, including two issues in 2007.[138,139] This also marked another change in direction for STH. Although mostly an issue-theme-driven publication, the move to primarily online access reduces the need to continue creating issues with specific themes and the more generic "Hot Topics" banner[139] was essentially the forerunner to the future "nonthemed" compilation issues that we continue to progress in 2023. In early 2008, Eberhard F Mammen, the founding EIC of STH, became increasingly unwell and eventually passed away,[140–142] and I was asked by the publisher to take over as EIC of STH, which I did, somewhat reluctantly at the time, given the commitment I knew this would require. The readership may not have noticed the unfolding turmoil that was STH at that time, being without the then EIC at the helm. I took over the reins unofficially in early 2008, guest editing five out of eight STH issues that year. I do not know how I managed to pull this off, but the journal survived that tumultuous year, and I took over officially as EIC in 2009. 2023 marks my 21st year of association with STH, and my 15th year as EIC. Eberhard Mammen was EIC for 34 years, from 1974 to 2008. It is unlikely that I or any other individual will ever match that record.

I am often asked how I manage to juggle all of my commitments, including that I am a full-time employee of NSW Health and also an EIC of STH. The main answer is very little down time. Most of my STH duties are completed in my own time—evenings, weekends, during my annual leave, and now increasing during my long service leave. And of course, I have a very understanding and patient wife and family.

Conclusion

I thank the readers who have managed to stick this out to the end. There has been much change in hemostasis testing and disease diagnostics in the last 30 to 40 years, and much of this evolution was mirrored by my own personal experience based at Westmead Hospital during this time. I suspect similar recounts are available from other long-lived "coagulationists," and I refer to the review from Robert Gosselin for an alternative recount from a U.S. perspective.[143]

Several hemostasis instruments have come and gone over time, with our own local experience reflected by the usage of the Coag-A-Mate X2, the ACL-300R, the MDA-180, the BCS XP, and several StaR Evolution analyzers. Some instruments remain in use today, with local experience reflected by the PFA-100, PFA-200, the AggRAM, the CS-5100, an AcuStar, a Hydrasys gel system, and two ACL-TOP 750s. Yes, our PFA-100 is still going strong, some 25 years after initial

installation, although the manufacturer is threatening to cease support of this model soon. We hope they don't!

Our laboratory still has a water bath, but this is primarily used to defrost frozen samples, and manual clotting tests are only performed as a teaching aide when the medical students visit. Like many others, the Westmead laboratory has migrated across several methodologies over the years. Laurel gel rockets, used for several assays in the 1980's, were replaced with ELISA assays and if tests maintained eventually placed on automated instruments. Some once popular procedures (e.g., skin bleeding times) were abandoned. Radioisotopic assays used in the 1980's were replaced by alternate safer methods or abandoned. Test numbers have increased markedly over time, with nearly 200,000 hemostasis tests now performed at the Westmead site every year. Thrombophilia assays were added to the test repertoires over time, as were tests to measure several anticoagulant drugs, in particular the DOACs. I hope my personal history, reflecting on the changes in hemostasis testing over my career to date in this field is found to be of interest to the readership, and I hope they forgive any inaccuracies I have inadvertently introduced in this reflection of the past.

Conflict of Interest
None declared.

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