Mini Review

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Towards 50 years of platelet function analyser (PFA) testing

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Abstract: The platelet function analyser (PFA) is a prevalent platelet function screening instrument, and comes in two models–the original PFA-100 and the contemporary PFA-200. The instruments have 'identical' output, being a 'closure time' (CT). Moreover, normal reference ranges provided by the manufacturer, for the specific test cartridges, are the same for both models. There are three different types of test cartridge: collagen/epinephrine (C/Epi), collagen/adenosine diphosphate (C/ADP), and "Innovance PFA P2Y" (only available in certain geographical locations). The PFA-100 was released in the mid 1990s, and so is approaching 50 years of age. The PFA-200, released in some locations in the mid 2010s, is destined to eventually replace the PFA-100, but is not yet available in the USA. The test system is highly sensitive to von Willebrand disease (VWD; C/Epi and C/ADP) and to aspirin therapy (C/Epi only), but only has moderate sensitivity to defects in platelet function and/or deficiencies in platelet number. Accordingly, recommendations for use for screening platelet function vary according to user experience. Some workers have alternatively used the PFA to assess thrombosis risk or pre-operative bleeding risk. In this review, we provide an overview of the history of PFA, and summarise its current clinical utility.

Keywords: *in vitro* bleeding time; PFA-100; PFA-200; platelet function analyser.

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Introduction

The platelet function analyser (PFA) is a widely popular platelet function screening instrument, and comes in two models–the original PFA-100 and the contemporary PFA-200. The PFA-100 was released into the marketplace in the mid 1990s [1–3], and so is approaching 50 years of age. The PFA-200, released in some locations, including Australia and Europe, in the mid 2010s, is destined to eventually replace the PFA-100, but is not yet available in some locations, notably in the USA. The PFA-100 was originally viewed as a replacement for the then 'popular' skin bleeding time, and was known for a while as the '*in vitro* bleeding time' [4–6].

The two instruments are treated as 'identical' in terms of test results, being a 'closure time' (CT) [7]. This CT is the time in seconds (s) taken for occlusion of an aperture in the test cartridge, after whole blood anticoagulated with buffered sodium citrate comes into contact with platelet aggregating agonists coated on a membrane in the cartridge. There are three different types of test cartridge, the two main ones: collagen/epinephrine (C/Epi) and collagen/adenosine diphosphate (C/ADP), and a third "Innovance PFA P2Y" only available in certain geographical locations (again, excluding the USA).

That the two instrument outputs are treated as 'identical' for both PFA-100/200 by the manufacturer (Siemens Healthineers) is clearly identifiable since the normal reference ranges (NRR) provided by the manufacturer with the different test cartridges are the same for both models. The current manufacturer-cited NRR for both the PFA-100 and PFA-200, and for the C/Epi and C/ADP test cartridges are respectively 82–150 s and 62–100 s, using standard 3.2% (0.105 M) sodium citrate blood collection tubes (Table 1).

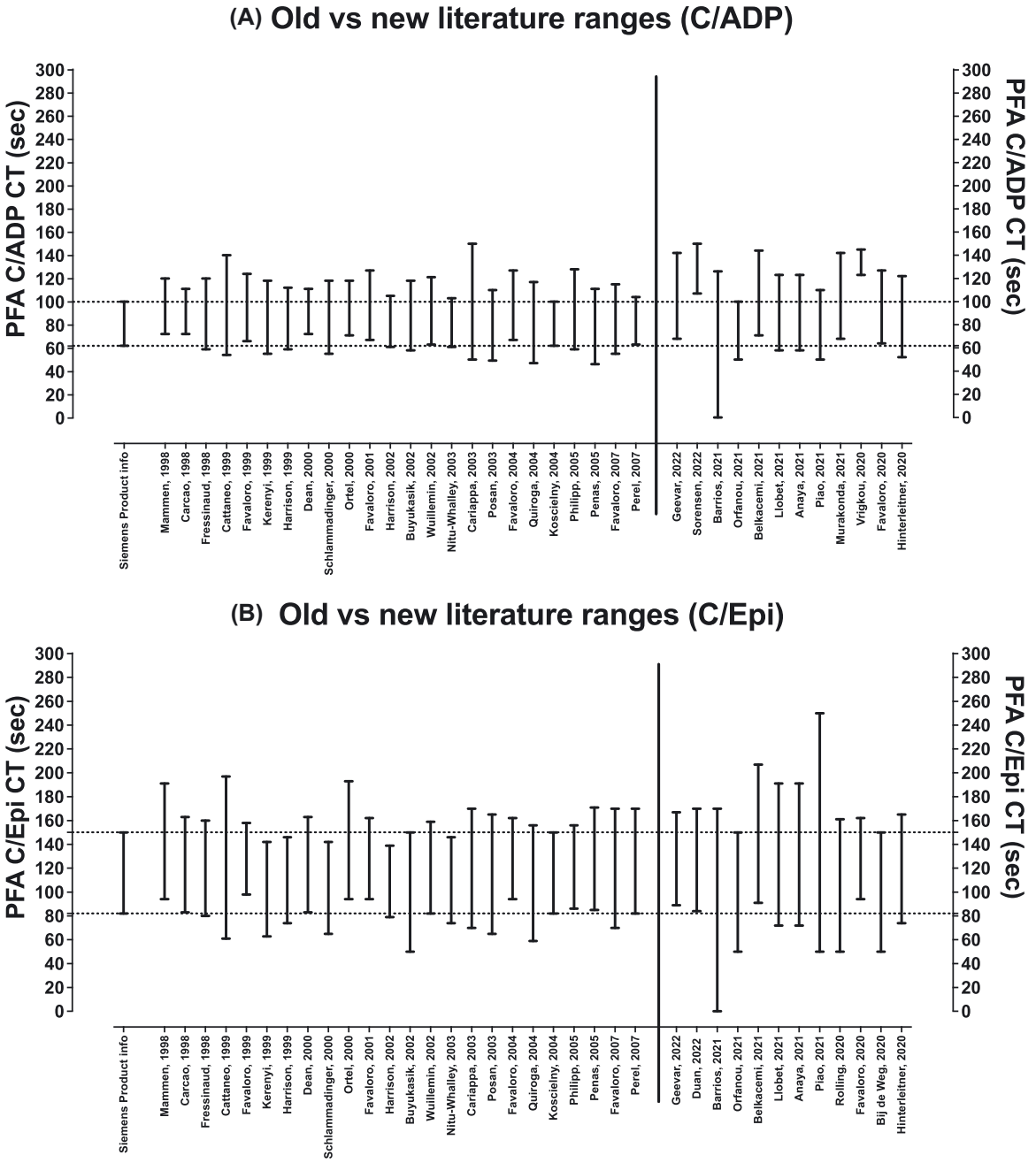

Figure 1: Normal reference range (NRR) data sets from the literature. Panels (A) and (B) respectively show some examples of published NRR data for C/ADP and C/Epi CTs, with some historical data detailed in a previous review [9] shown on the left and more contemporary data detailed in a recent publication [25] shown on the right. The Siemens product information NRR CTs are shown by the horizontal dashed lines. The historical and contemporary data show similar variations in NRRs. Most literature data, both historical and contemporary, approximate the manufacturer indicated ranges for C/Epi, but tend to be much wider than the manufacturer ranges for C/ADP.

The PFA-100/200 test system, using C/Epi and C/ADP cartridges, is highly sensitive to von Willebrand disease (VWD) and to aspirin therapy (C/Epi only), but has only moderate sensitivity to defects in platelet function and/or deficiencies in platelet number [7–12]. Accordingly, recommendations for their use as a platelet function screening instrument vary according to user experience. For example, the Platelet Physiology Subcommittee of the Scientific and Standardization Committee (SSC) of the International Society on Thrombosis and Haemostasis

Table 1: Some early and some contemporary reported normal reference ranges (NRR) for the PFA.

Data from	No. donors (Col/ADP, Col/Epi)	Col/ADP, s	Col/Epi, s
Current manufacturer NRR (PFA-100 and PFA-200) 3.2% citrate	309	62–100	82–150
Current manufacturer NRR (PFA-100 and PFA-200) 3.8% citrate	127	68–121	84–160
Original publication from Kundu et al. [1]	28–35	63–123	97–188
Early 'field study' from Mammen et al. [3] (3.8% citrate)	99	77–133	98–185
Later updated 1998 study from Mammen et al. [18] (3.8% citrate)	206	72–120	94–191
Recent network evaluation [25] NRR in use in each site			
Site 1		64–127	94–162
Site 2		73–127	94–162
Sites 3&4 (harmonised)		60–120	80–170
Site 5		73–127	81–146
Site 6		71–125	94–193
Site specific NRRs (based on site specific normal donor data, excluding outliers; 2.5th–97.5th percentiles)			
Site A	48, 47	75–124	97–157
Sites B&C (combined data)	46, 46	67–116	92–160
Site D	42, 38	65–123	89–151
Site E	38, 33	65–116	84–168
Site F	22, 20	69–142	75–181
Composite NSWHP NRR (excluding outliers)	194, 184	69–124	89–160

NRR, normal reference range; NSWHP, New South Wales Health Pathology; PFA, platelet function analyser.

(ISTH) reflected in 2006 that "the test does not have sufficient sensitivity or specificity to be used as a screening tool for platelet disorders" [12]. We essentially agree with this view, and although the C/EPI and C/ADP CTs have utility for exclusion of severe platelet dysfunction, they have insufficient sensitivity to exclude mild platelet function disorders, which account for the majority of such disorders, thereby limiting the utility of both PFA-100 and PFA-200. Moreover, the P2Y cartridge, whilst marketed by the manufacturer as sensitive to clopidogrel, has in our experience poor utility for same. However, the PFA100/200 is very sensitive to von Willebrand factor (VWF) level and activity, and thus has a role in screening for von Willebrand disease (VWD) [7–11]. Some of us have also shown the potential utility of these instruments in monitoring certain therapies in VWD, in particular desmopressin (DDAVP) use [13, 14]. Other workers have alternatively used the instrument to assess thrombosis risk or pre-operative bleeding risk [15–17]. We therefore provide an overview of the history of the PFA, and its clinical utility.

Early historical aspects of the PFA and its use

As mentioned, the PFA-100 was released into the marketplace in the mid 1990s [1–3], as largely accompanied by two seminal papers [2, 3] published in a supplement to the journal *Seminars in Thrombosis and Hemostasis (STH)*. One paper provided a detained description of the PFA-100 [2], and was authored by employees of the manufacturer (then Dade Diagnostics). The other paper provided early data from a field trial of PFA-100 [3], and was authored by the then Editor in Chief of that journal, Prof. Eberhard Mammen and his colleagues. A third article from Mammen and colleagues was published in STH in 1998 [18]. As noted in a separate publication [19], two of these papers [2, 18] count among the five most highly cited papers from STH from all time. Notably, the PFA-100 was developed from an earlier instrument called the Thrombostat 4,000, with descriptions of this instrument and its utility taking up the majority of the aforementioned supplement to STH. We mention this history because it reflects an interesting chronicle, which also helps define the then potential future utility of the PFA-100. Thus, the Thrombostat 4000 was identified in that STH supplement as a sensitive screening test for VWD [20], a useful method to test antiplatelet agents (then primarily aspirin) [21], and being affected by various hematological parameters [22], with all these also shown to later hold true for the PFA-100 [7–9]. The Thrombostat 4000 was also described as an *in vitro* bleeding time [23, 24], which was also adopted as a descriptor for the PFA-100 [4–6].

In summary, in the initial years of the PFA-100, its use developed as a replacement of the more invasive and less standardized 'skin bleeding time', as well as replacing the larger, less user friendly Thrombostat 4000, and for screening of platelet function and VWD, as well as a test for evaluation of antiplatelet agents, most notably aspirin.

What is the normal reference range for the PFA-100 or PFA-200?

One of the more interesting historical aspects of the PFA-100 and PFA-200 is the question of the NRR for tests

performed on the instrument. As noted above, the current manufacturer-cited NRR for the C/Epi and C/ADP test cartridges are respectively 82–150 s and 62–100 s, for use on both the PFA-100 and PFA-200 using the standard 3.2% (0.105 M) sodium citrate blood collection tubes (and based on 309 normal donors). The corresponding NRR for use of 3.8% (0.129 M) citrate are 84–160 s (C/Epi) and 68–121 s (C/ADP) (based on 127 donors). However, the first description of the PFA-100, in a meeting abstract [1], actually listed the NRR as 97–188 s (C/Epi) and 63–123 s (C/ADP) (as based on 28–35 donors; citrate concentration not specified, but presumed to be 3.8%). In the separate independent field trial reported by Mammen and colleagues [3], the derived NRR based on 99 normal donors were 77–133 s (C/ADP) and 98–185 s (C/Epi), as based on 3.8% citrate. In the later updated study [18], the estimated NRR were reported as 72–120 s (C/ADP) and 94–191 s (C/Epi), again based on 3.8% citrate. These early variations are listed in Table 1.

In an early review from one of us [9], it was identified that this variation in reported NRRs continued for the next decade, and in a recent update, can be shown to continue to the current day [25]. Figure 1 shows a summary of some of this variation, which even includes variation within laboratories belonging to the same pathology network (Table 1). The manufacturer states in its product information: "As differences in donor population and other factors may affect results, it is recommended that each laboratory demonstrates transference of the reference ranges shown above (refer to CLSI guideline C28-A324 [26]). If transference of these reference range cannot be verified, the laboratory should establish its own reference ranges." Although this is standard laboratory practice, we can highlight some problems with this approach.

First, in a recent NSW Health Pathology (NSWHP) Network evaluation [25], marked differences were seen in both the established NRRs, as used throughout the network, as well as those NRRs that could be generated using updated site-specific normal donor samples (Table 1 [25]). We would not expect to see such different NRRs based on different populations, since populations at each site location would be expected to be similar. Similarly, the differences would not be based on differences in collection tubes, since all sites use 3.2% citrate. Most likely, the different NRRs determined at each site just reflect the use of a different, perhaps too small in number, group of normal individuals, who may display variable effects of factors that influence the CT (such as platelet count, hematocrit value, VWF level and activity, prevalence of blood groups, and potential use of recent medication or ingestion of certain foods or supplements [9]). However, the cut-off value representing the upper limit of the NRR will generally be used to dichotomise individual CTs as 'normal' (below or equal to upper cut-off value) or 'abnormal' (above upper cut-off value). Thus, the NRR estimated by laboratories will determine whether a patient result is classified as normal or abnormal, and thus whether a patient may be identified to have a primary hemostasis defect or not. Clearly, too high a cut-off value will miss patients with hemostasis defects, and too low a cut-off value will over-call patients as suffering hemostasis defects. Thus, the different arising NRRs in our network would mean that should the same individual be assessed at different sites, some sites might classify that individual as normal, and others as not.

Many supplements and drugs may affect the CT, and even if the usual 'culprits' (e.g., non-steroidal anti-inflammatory drugs such as aspirin) are excluded in selected normal donors, others may not be recognised. Even certain foods can affect CTs, for example high chocolate and garlic intake [9]. In the NSWHP study [25], we were able to determine that all sites could adopt C/Epi ranges from the manufacturer, based on transference using CLSI guidance [26], and as per manufacturer advice, as based on the fact that >90% of data points fell within the manufacturer ranges. However, no site could adopt the C/ADP ranges from the manufacturer, which are much tighter with a very low comparative cut-off of 100 s. Indeed, a similar problem is found with the NRR reported in the literature (Figure 1; [25]). Thus, there is closer alignment in literature NRR data to the manufacturer C/Epi CT than to the C/ADP CT. This suggests that the manufacturer NRR for C/ADP may require a reset, as laboratories that adopt this NRR may over call patients as having an abnormal C/ADP CT.

One historical article published by one of us more than 20 years ago failed to find any significant difference of both C/Epi and C/ADP CT in pediatrics, showing just a non-significant trend towards lower values in children up to 15 years old [27]), likely reflecting developmental hemostasis and progressive increase of VWF in parallel with age [28, 29]. Moreover, there are no known differences in CT between sexes, or in older people [29], despite increasing levels of VWF as we age [30].

What is the clinical utility of the PFA-100 and PFA-200?

A recent PubMed search of "(PFA 100) OR (PFA 200) OR (platelet function analyser)" yielded 2,554 results, although not all captured papers actually reflect use of the PFA-100 or PFA-200. Indeed, in the literature, the term 'PFA' has also been used to abbreviate terms such as

'pulsed-field ablation', 'parallel factor analyses', 'profunda femoris artery', 'parameter-free attention', 'patellofemoral arthroplasty', and 'paraformaldehyde' to name a few. Nevertheless, the majority of captured papers do reflect use of the PFA-100 or PFA-200, with ongoing similar results over recent years, averaging around 100–140 papers/year in recent times (Figure 2), and suggesting that we will not see any expected lack of interest in this analyser in the foreseeable future.

The utility of the PFA-100/200 has been explored in several reviews from the lead author [7–9], with a brief summary of potential applications detailed in Table 2.

What is the evidence for 'equivalence' of the PFA-100 and PFA-200?

Given the age of the PFA-100 technology, the manufacturer has replaced the PFA-100 with the newer model, the PFA-200, in some geographic locations such as Australia and Europe. In theory, the mechanical elements of the two instruments are the same, with the PFA-200 essentially updating the user interface (touch screen), updating the software, and increasing quality control requirements (e.g., forced daily system checks). So, what is the evidence

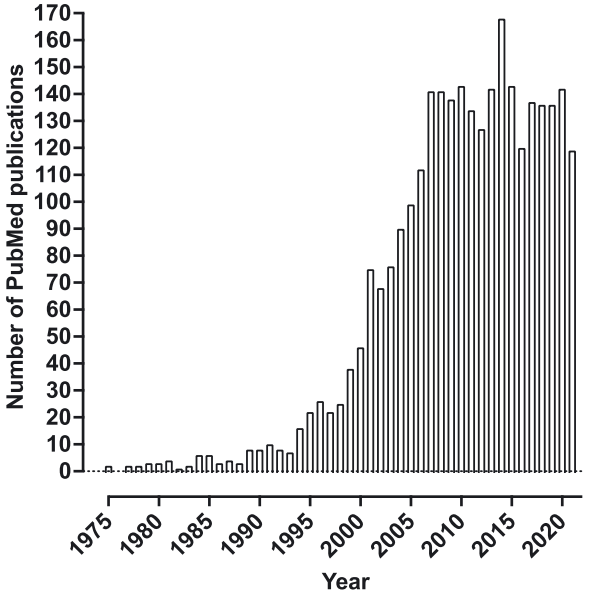

Figure 2: PubMed publications over the years using a search of "(PFA 100) OR (PFA 200)) OR (platelet function analyser)". Similar results are evident in the contemporary literature on a year by year basis, averaging around 100–140 papers/year.

Table 2: A summary of the proposed utility of the PFA-100/200 from the literature.[a]

Proposed utility	Examples
Excellent screen for von Willebrand disease (VWD)	Normal PFA closure times (CTs) provide excellent negative predictive value (i.e. absence of severe VWD), sometimes better than individual components of VWF assays [10, 11]
Early aid to assessment of utility of desmopressin (DDAVP) therapy in VWD patients, noting that PFA CTs can usually be obtained earlier than VWF test values	Patterns of findings combining PFA CTs with VWF test parameters post DDAVP show that normalisation of VWF activity levels often accompanied by normalisation of CTs. Thus, normalisation of CTs post DDAVP is highly suggestive of normalisation of VWF activity levels [13, 14]
Aid to VWD type diagnosis following desmopressin (DDAVP) therapy	Patterns of findings combining PFA CTs with VWF test parameters post DDAVP can provide additional evidence for a particular VWD type in patients where VWD type is uncertain [13, 14]
Aid to assessment of presurgical bleeding risk	As a replacement for the 'skin bleeding time', normal CTs can be used as a marker of low surgical bleeding risk [17]
Assessment of thrombotic risk	Short CTs as a risk factor for thrombosis or a marker of thrombophilia; also used in relation to potential 'failure' of aspirin therapy [15, 16]
Assessment of drug therapy, drug effects, or other agents	Primarily aspirin, but also clopidogrel, nonsteroidal anti-inflammatory drugs (NSAIDs), anesthetics, antithrombotics and other compounds [9]
Assessment of blood expander/replacement agents	Including dextran sulfate, colloids, and crystalloids; also for quality control of platelet concentrates [9]
Assessment of herbal medicines or extracts or dietary agents	Various flavonoids, *Ginkgo biloba*, garlic, asian ginseng, St. John's wort, saw palmetto, chocolate/cocoa, *p*-coumaric acid (a common dietary phenol) [9]
Animal studies	Primarily dogs, but also including camels, horses, pigs, and hamsters; studies have included assessment of aspirin effects (dogs), cardiac disease (dogs), pollution (hamsters), anesthetics (pigs), and the identification of a case of Glanzmann thrombasthenia (horse) [9]

[a]Mostly summarised and updated from [9].

that the output from the PFA-100 and the PFA-200, namely the CTs, are identical? Interestingly, there are no head to head studies of PFA-100 vs. PFA-200 that we could identify from the literature, other than a study from the Westmead laboratory [25], and some external quality assessment (EQA) data which also in part comes from some of us [31].

In summary, there are a number of published evidences that the CTs from the PFA-100 and PFA-200 are 'identical'. First, as mentioned, the manufacturer defined NRRs for the PFA-100 and PFA-200 are the same, and align instead to the test cartridges used. Accordingly, the manufacturer considers the output from the PFA-100 and PFA-200 as essentially equivalent. Second, a direct comparison of PFA-100 vs. PFA-200 CTs has been performed by the Westmead laboratory to verify their equivalence for laboratory diagnostic use, and for accreditation purposes [25]. Third, EQA data from the Royal College of Pathologists of Australasia Quality Assurance Program (RCPAQAP) has captured data related to PFA-100 and PFA-200 use by EQA participants, and sub-analysis of data for PFA-100 vs. PFA-200 did not uncover any concerning differences [31]. Finally, as the PFA-200 starts to take hold in laboratories, one can assess if there are any concerning signals in the literature related to different findings, which are not evident to our knowledge. Indeed, in both the Westmead and Verona laboratories, we continue to use the PFA-200 for the same purpose and in the same way as the PFA-100, with essentially equivalent findings.

The PFA-100/200 and *CCLM*

As noted elsewhere, this journal is celebrating its 60th birthday. *Clinical Chemistry and Laboratory Medicine* (*CCLM*) has been part of the landscape for about 10 years longer than the PFA-100. Nevertheless, as often happens in a journey spanning 60 years, there are intertwined histories, and several relevant papers related to the PFA-100, or to related fields of platelet function and VWD screening and testing have been published in *CCLM* over past decades. The most relevant papers are listed in Table 3, together with a brief description of the importance to the field.

Table 3: Select papers published in CCLM related to the PFA-100/200 and/or related areas.

Authors, date[a]	Key findings	Comments
PFA-100/200		
Golanski et al., 2004 [32]	Evaluated the usefulness of the PFA-100 (C/ADP, C/EPI cartridges) to assess the *in vitro* effects of a few platelet function inhibitors on platelet function, as compared with the more commonly used diagnostic technique based on whole blood electrical aggregometry. Findings point to a limited usefulness of the C/ADP, C/EPI cartridges for the monitoring of the effectiveness of ADP receptor antagonists.	Similar findings reported by others. Siemens eventually released their Innovance PY2 cartridge for potential use in monitoring of P2Y12 inhibitors.
Stegnar et al., 2007 [33]	Examined the PFA-100 for its usefulness in assessing the efficacy of platelet membrane glycoprotein IIb/IIIa antagonists *in vitro*, as compared to optical platelet aggregometry. Authors concluded that CTs represented a fast, simple and sensitive method of assessing glycoprotein IIb/IIIa antagonism *in vitro*, is comparable to optical aggregometry, and suitable for testing larger numbers of glycoprotein IIb/IIIa antagonists.	Recognised sensitivity of PFA-100 C/ADP and C/Epi cartridges to glycoprotein IIb/IIIa antagonists eventually led to use of these antagonists for use in EQA exercises ('wet challenges') by US CAP.
Wallin et al., 2008 [34]	Evaluated the effect of pneumatic tube transport on hematology and coagulation parameters, including platelet function with PFA-100. No preanalytical effect of pneumatic tube transport could be seen for most haematology and coagulation parameters, as well as analysis with PFA-100.	It is now recognised that pneumatic tube transport can affect PFA-100 CTs, perhaps depending on the transport system in use. At the Westmead site, we observe considerable shortening of PFA-100 CTs in samples sent through the pneumatic tube system, which is recognised as a reason for rejection of samples and recollection
Favaloro and Bonar, 2012 [35]	Described a novel approach to EQA for the PFA-100/200, including wet challenges and test interpretation. Reported CTs for each challenge were within limits of expectation and good reproducibility was evidenced by repeated challenges. Coefficients of variation (CVs) generated by challenges were similar to those obtained using native whole blood and interpretations were in general also consistent with expectations and test data provided by laboratories.	This external quality assessment group (the RCPAQAP) has been providing EQA material for PFA-100/200 challenges since 2008, as produced by the lead author of this review, and as evidencing the feasibility of EQA for platelet function testing. These EQA challenges are now also distributed by ECAT for its EQA program.

Table 3: (continued)

Authors, date[a]	Key findings	Comments
Favaloro, 2013 [36]	Described a novel approach to internal quality control (IQC) for the PFA-100/200, based on similar concepts to that used in the preceding study related to EQA. Thus, the same wet challenge process for EQA, can also be utilised to extend the process to IQC.	This study provided the first evidence of feasibility, or proof of concept, for IQC testing for the PFA-100 incorporating pathological test findings and Levey-Jennings plots. Such a concept is also potentially more broadly applicable to other platelet function, or whole blood or cell-based test systems
Eller et al., 2014 [37]	Assessed the effect of several anticoagulants (argatroban, dabigatran, rivaroxaban, apixaban, fondaparinux) on platelet function tests, including the PFA-100. None of the drugs seemed to have any influence on PFA or multiplate test results.	It is now well known that PFA-100/200 CTs are not affected by a wide variety of anticoagulants. This study extended previous findings related to heparin and vitamin K antagonists such as warfarin.
Fierro et al., 2016 [38]	Assessed the prevalence of hemostatic abnormalities in patients with spontaneous, recurrent subconjunctival hemorrhage (SCH) to clarify the role of the hemostasis laboratory in this clinical setting, and including PFA-100 CTs and von Willebrand factor tests. Authors concluded that the prevalence of hemostatic alterations in patients with recurrent, spontaneous SCH was no different from the general population; thus, hemostatic screening or second level tests are not indicated in patients with recurrent SCH and no other bleedings.	The PFA-100/200 isn't going to always be clinical useful in all settings. This study showed one application for which the PFA-100/200 is not likely to be useful.
Valsami, 2020 [39]	Evaluated PFA-100 CTs in cord blood samples of healthy term and preterm neonates, and defined normal reference ranges (NRR) for these.	Given the difficulties in collecting sufficient blood from neonates, cord blood becomes a default sample for hemostasis evaluations. This study provided some data on C/EPI CT ranges for healthy term neonates and for healthy preterms.
Rolling et al., 2020 [40]	Compared aspirin and clopidogrel 'non-responsiveness' using light transmittance aggregometry (LTA) vs. PFA-100® in patients undergoing neuroendovascular procedures, concluding that the differences in test results highlight that LTA and PFA-100® are not interchangeable.	Perhaps fitting that the last manuscript published in CCLM on PFA-100/200, at the time of writing this historical review, returns to a similar topic to the first paper published in CCLM on PFA-100 (see above (also reference 1 below).
Platelet function (other)		
Thijs et al., 2010 [41]	Authors reviewed the current knowledge of platelet intracellular signal transduction pathways involved in platelet adhesion, activation, amplification of the activation signal and aggregation, as well as pathways limiting platelet aggregation.	Potentially linked to PFA-100/200 given its dependence on platelet function.
Favaloro et al., 2010 [42]	A previous review from some of the authors to the current review that outlines the sequential process of platelet function investigations, and discusses each of the essential components in some detail.	Also mentions the place of the PFA-100/200 in the setting of platelet function investigations.
Stegnar, 2011 [43]	Investigated the effect of several pre-analytical variables on light transmittance aggregometry, otherwise considered the gold standard for platelet function testing. Blood collection and plasma preparation can be simplified by omitting short-term stabilization of PRP and adjustment for platelet count. The subjects can be tested from morning to mid-day, and a light breakfast is acceptable. However, the analyses should not be postponed for more than 2 h if arachidonic acid or ADP are used as agonists.	Essentially reflects the practice undertaken at westmead.
Mani et al., 2011 [44]	Evaluated platelet function testing in hirudin and BAPA (a dual thrombin/factor Xa inhibitor benzylsulfonyl-D-Arg-Pro-4-amidinobenzylamide) anticoagulated blood compared to standard sodium citrate. Authors found that use of hirudin or BAPA anticoagulated blood resulted in improvement of stability of platelet function measurements.	Hirudin is a common anticoagulant now used routinely for platelet aggregation testing using the Multiplate analyser.
Andreasen et al., 2014 [45]	Investigated whether the size of sample tubes affected whole blood coagulation and platelet function analyses, using rotational thromboelastometry (ROTEM) and multiplate analysers. Platelet aggregation was significantly lower when using smaller	Potentially linked to PFA-100/200 given its dependence on platelet function.

Table 3: (continued)

Authors, date[a]	Key findings	Comments
	tubes; thus different size tubes cannot be used interchangeably.	
Carubbi et al., 2014 [46]	A review that discusses the laboratory approach for the differential diagnosis of the most common inherited platelet disorders, suggesting a common multistep flowchart model which starts from the simpler test (platelet count) ending with the more selective and sophisticated analyses.	A useful follow up review to the review by Favaloro et al. as mentioned above (see also reference 11 below).
von Willebrand factor or von Willebrand disease		
Meiring et al., 2007 [47]	Evaluated an in-house VWF collagen-binding assay using type III collagen, using 44 patients with VWD and 40 normal subjects, and with other assays including VWF antigen, ristocetin cofactor activity, ristocetin-induced platelet agglutination and VWF multimeric analysis. The cost of the in-house collagen-binding assay was 10-fold lower than that of commercial kits, and had acceptable precision, and was shown to be sensitive to large VWF multimers.	It is now well known that the PFA-100/200 CTs correlate well with levels of VWF, in particular functionally active VWF, especially as assessed using a VWF collagen-binding assay.
Franchini et al., 2014 [48]	In this review, the authors focus on the molecular mechanisms underlying the interaction between ABO blood group and VWF in normal and pathological conditions.	It is now well known that the ABO blood group influences the level of plasma VWF, which in turn is a main driver of PFA-100 CTs.

C/ADP, collagen/adenosine diphosphate; C/Epi, collagen/epinephrine; CT, closure time; ECAT, External quality Control of diagnostic Assays and Tests (with a focus on Thrombosis and Haemostasis); EQA, external quality assessment; US CAP, United States College of Pathologists; RCPAQAP, Royal College of Pathologists of Australasia Quality Assurance Program. [a]References as listed below.

Conclusions

The PFA-100 has been part of the laboratory landscape for nearly 50 years. The PFA-100 is gradually being replaced by a newer model called the PFA-200, but both instruments yield essentially the same test results, being a closure time, and using different test cartridges, with differing sensitivities to various parameters such as platelet count and function, VWF level and activity, anti-platelet medication, and various foods and supplements. The PFA-100/200 is used by different laboratories for potentially different reasons, but at its heart, the high sensitivity to disturbances in VWF level and function suggests its main purpose is for rapid exclusion of VWD, should normal CTs be determined with C/ADP and C/Epi cartridges [10, 11].

In turn, as *CCLM* is turning 60, the story of the PFA-100/200, or associated papers in *CCLM* (Table 3), indicates at least some shared history. The authors wish both the PFA-100/200 and *CCLM* the best of wishes for their respective 50- and 60-year anniversaries.

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References

1. Kundu S, Sio R, Mitu A, Ostgaard R. Evaluation of platelet function by PFA-100. Clin Chem 1994;40:1827–8.
2. Kundu SK, Heilmann EJ, Sio R, Garcia C, Davidson RM, Ostgaard RA. Description of an in vitro platelet function analyzer–PFA-100. Semin Thromb Hemost 1995;21:106–12.
3. Mammen EF, Alshameeri RS, Comp PC. Preliminary data from a field trial of the PFA-100 system. Semin Thromb Hemost 1995;21: 113–21.
4. Van der Planken MG, Claeys MJ, Vertessen FJ, Dilling D, Bosmans JM, Berneman ZN, et al. Comparison of turbidimetric aggregation and in vitro bleeding time (PFA-100) for monitoring the platelet inhibitory profile of antiplatelet agents in patients undergoing stent implantation. Thromb Res 2003;111: 159–64.
5. Troost MM, van Genderen PJ. Excessive prolongation of the Ivy bleeding time after aspirin in essential thrombocythemia is also demonstrable in vitro in the high shear stress system PFA-100. Ann Hematol 2002;81:353–4.

6. Sap F, Kavaklı T, Kavaklı K, Dizdarer C. The prevalence of von Willebrand disease and significance of in vitro bleeding time (PFA-100) in von Willebrand disease screening in the izmir region. Turk J Haematol 2013;30:40–7.
7. Favaloro EJ. Clinical utility of closure times using the platelet function analyzer (PFA)-100/200. Am J Hematol 2017;92:398–404.
8. Favaloro EJ. Clinical application of the PFA-100®. Curr Opin Hematol, 2002;9:407–15.
9. Favaloro EJ. Clinical utility of the PFA-100. Semin Thromb Hemost 2008;34:709–33.
10. Ardillon L, Ternisien C, Fouassier M, Sigaud M, Lefrançois A, Pacault M, et al. Platelet function analyser (PFA-100) results and von Willebrand factor deficiency: a 16-year 'real-world' experience. Haemophilia 2015;21:646–52.
11. Favaloro EJ. Utility of the platelet function analyser (PFA-100/200) for exclusion or detection of von Willebrand disease: a study 22 years in the making. Thromb Res 2020;188:17–24.
12. Hayward CP, Harrison P, Cattaneo M, Ortel TL, Rao AK. Platelet physiology subcommittee of the scientific and standardization committee of the international society on thrombosis and haemostasis. platelet function analyzer (PFA)-100 closure time in the evaluation of platelet disorders and platelet function. J Thromb Haemostasis 2006;4:312–9.
13. Favaloro EJ, Thom J, Patterson D, Just S, Baccala M, Dixon T, et al. Potential supplementary utility of combined PFA-100 and functional VWF testing for the laboratory assessment of desmopressin and factor concentrate therapy in von Willebrand disease. Blood Coagul Fibrinolysis 2009;20:475–83.
14. Favaloro EJ, Kershaw G, Bukuya M, Hertzberg M, Koutts J. Laboratory diagnosis of von Willebrand Disorder (VWD) and monitoring of DDAVP therapy: efficacy of the PFA-100® and VWF:CBA as combined diagnostic strategies. Haemophilia 2001;7:180–9.
15. Vazquez-Santiago M, Vilalta N, Cuevas B, Murillo J, Llobet D, Macho R, et al. Short closure time values in PFA-100 are related to venous thrombotic risk. Results from the RETROVE study. Thromb Res 2018;169:57–63.
16. Llobet D, Vallvé C, Tirado I, Vilalta N, Carrasco M, Oliver A, et al. Platelet hyperaggregability and venous thrombosis risk: results from the RETROVE project. Blood Coagul Fibrinolysis 2021;32:122–31.
17. Koscielny J, Ziemer S, Radtke H, Schmutzler M, Pruss A, Sinha P, et al. A practical concept for preoperative identification of patients with impaired primary hemostasis. Clin Appl Thromb Hemost 2004;10:195–204.
18. Mammen EF, Comp PC, Gosselin R, Greenberg C, Hoots WK, Kessler CM, et al. PFA-100 system: a new method for assessment of platelet dysfunction. Semin Thromb Hemost 1998;24:195–202.
19. Favaloro EJ. Towards 50 years of seminars in thrombosis and hemostasis. Semin Thromb Hemost 2022;8. in press.
20. Weippert-Kretschmer M, Witte M, Budde U, Vigh T, Kretschmer V, Scharrer I. The Thrombostat 4000. A sensitive screening test for von Willebrand's disease. Semin Thromb Hemost 1995;21:44–51.
21. Kratzer MA, Negrescu EV, Hirai A, Yeo YK, Franke P, Siess W. The Thrombostat system. A useful method to test antiplatelet drugs and diets. Semin Thromb Hemost 1995;21:25–31.
22. Söhngen D, Hattstein E, Heyll A, Meckenstock G, Wienen S, Schneider W. Hematological parameters influencing the Thrombostat 4000. Semin Thromb Hemost 1995;21:20–4.
23. lshameeri RS, Mammen EF. Performance characteristics and clinical evaluation of an in vitro bleeding time device–Thrombostat 4000. Thromb Res 1995;79:275–87.
24. Högman CF, Eriksson L, Kristensen J. Leukocyte-depleted platelets prepared from pooled buffy coat post-transfusion increment and "in vitro bleeding time" using the Thrombostat 4000/2. Transfus Sci 1993;14:35–9.
25. Favaloro EJ, Mohammed S, Vong R, Chapman K, Kershaw G, Just S, et al. Harmonizing platelet function analyzer testing and reporting in a large laboratory network. Int J Lab Hematol 2022. https://doi.org/10.1111/ijlh.13907.
26. Clinical and Laboratory Standards Institute (CLSI). Defining, establishing, and verifying reference intervals in the clinical laboratory; approved guideline - third edition. CLSI document C28-A3, CLSI, 940 West Valley Road, Suite 1400, Wayne, PA 19087-1898 USA, 2008.
27. Lippi G, Manzato F, Franchini M, Brocco G, Florenziani G, Guidi G. Establishment of reference values for the PFA-100 platelet function analyzer in pediatrics. Clin Exp Med 2001;1:69–70.
28. Lippi G, Franchini M, Montagnana M, Guidi GC. Coagulation testing in pediatric patients: the young are not just miniature adults. Semin Thromb Hemost 2007;33: 816–20.
29. Sestito A, Sciahbasi A, Landolfi R, Maseri A, Lanza GA, Andreotti F. A simple assay for platelet-mediated hemostasis in flowing whole blood (PFA-100): reproducibility and effects of sex and age. Cardiologia 1999;44:661–5.
30. Favaloro EJ, Soltani S, McDonald J, Grezchnik E, Easton L, Favaloro JWC. Reassessment of ABO-blood group, gender and age on laboratory parameters used to diagnose von Willebrand Disorder (VWD): potential influence on the diagnosis versus the potential association with risk of thrombosis. Am J Clin Pathol 2005;124:910–7.
31. Favaloro EJ, Bonar R. An update on quality control for the PFA-100/PFA-200. Platelets 2018;29:622–7.
32. Golanski J, Pluta J, Baraniak J, Watala C. Limited usefulness of the PFA-100 for the monitoring of ADP receptor antagonists–in vitro experience. Clin Chem Lab Med 2004;42:25–9.
33. Stegnar M, Bozic M, Dolenc MS, Anderluh PS, Kikelj D. Utility of PFA-100 closure time vs. optical aggregometry in assessing the efficacy of platelet membrane glycoprotein IIb/IIIa antagonists in vitro. Clin Chem Lab Med 2007;45:1542–8.
34. Wallin O, Söderberg J, Grankvist K, Jonsson PA, Hultdin J. Preanalytical effects of pneumatic tube transport on routine haematology, coagulation parameters, platelet function and global coagulation. Clin Chem Lab Med 2008;46:1443–9.
35. Favaloro EJ, Bonar R. Proficiency testing/external quality assurance for the PFA-100(®). Clin Chem Lab Med 2012;50: 1393–401.
36. Favaloro EJ. Time for a conceptual shift in assessment of internal quality control for whole blood or cell-based testing systems? An evaluation using platelet function and the PFA-100 as a case example. Clin Chem Lab Med 2013;51:767–74.
37. Eller T, Busse J, Dittrich M, Flieder T, Alban S, Knabbe C, et al. Dabigatran, rivaroxaban, apixaban, argatroban and fondaparinux and their effects on coagulation POC and platelet function tests. Clin Chem Lab Med 2014;52:835–44.
38. Fierro T, Bartolini A, Mezzasoma AM, Guglielmini G, Falcinelli E, Orsini S, et al. Prevalence of hemostatic alterations in patients with recurrent spontaneous subconjunctival hemorrhage. Clin Chem Lab Med 2016;54:97–103.
39. Valsami S, Kollia M, Mougiou V, Sokou R, Isaakidou E, Boutsikou M, et al. Evaluation of PFA-100 closure times in cord

blood samples of healthy term and preterm neonates. Clin Chem Lab Med 2020;58:e113–6.
40. Rolling CC, Tomada J, Frölich AM, Holst B, Holstein K, Voigtländer M, et al. Comparison of acetylsalicylic acid and clopidogrel non-responsiveness assessed by light transmittance aggregometry and PFA-100® in patients undergoing neuroendovascular procedures. Clin Chem Lab Med 2020;59: 383–92.
41. Thijs T, Nuyttens BP, Deckmyn H, Broos K. Platelet physiology and antiplatelet agents. Clin Chem Lab Med 2010;(Suppl 1):S3–13.
42. Favaloro EJ, Lippi G, Franchini M. Contemporary platelet function testing. Clin Chem Lab Med 2010;48:579–98.
43. Stegnar M, Knezevic A, Bozic-Mijovski M. The effect of pre-analytical variables on light transmittance aggregometry in citrated platelet-rich plasma from healthy subjects. Clin Chem Lab Med 2010;48:1463–5.
44. Mani H, Hellis M, Lindhoff-Last E. Platelet function testing in hirudin and BAPA anticoagulated blood. Clin Chem Lab Med 2011;49:501–7.
45. Andreasen JB, Pistor-Riebold TU, Knudsen IH, Ravn HB, Hvas AM. Evaluation of different sized blood sampling tubes for thromboelastometry, platelet function, and platelet count. Clin Chem Lab Med 2014;52:701–6.
46. Carubbi C, Masselli E, Nouvenne A, Russo D, Galli D, Mirandola P, et al. Laboratory diagnostics of inherited platelet disorders. Clin Chem Lab Med 2014;52:1091–106.
47. Meiring M, Badenhorst PN, Kelderman M. Performance and utility of a cost-effective collagen-binding assay for the laboratory diagnosis of Von Willebrand disease. Clin Chem Lab Med 2007; 45:1068–72.
48. Franchini M, Crestani S, Frattini F, Sissa C, Bonfanti C. ABO blood group and von Willebrand factor: biological implications. Clin Chem Lab Med 2014;52:1273–6.