New guide to whole blood viscoelastic assays: hemostasis, testing, cases, and applications

New this month from CAP Publications is Whole Blood Viscoelastic Assays in Clinical Diagnosis: An Illustrated Case-Based Guide. *Viscoelastic testing was designed to determine the cause of intraoperative or trauma-related bleeding to guide hemostatic therapy.* CAP TODAY *asked the book's editor, Oksana Volod, MD, about the guide. Her answers follow, and a sample chapter begins on this page.*

Dr. Volod is professor of pathology and director of the coagulation consultative service, Cedars-Sinai Medical Center, Los Angeles.

Are whole blood viscoelastic assays in common use today?

The invention of thromboelastography (TEG), the first viscoelastic assay (VEA), predates the description of the activated partial thromboplastin time test in 1953, originating in Germany in 1948. However, widespread adoption of VEA in clinical laboratories occurred only after the introduction of a cell-based model of hemostasis in 1994, which emphasized the crucial roles of platelets and tissue factor in hemostasis. Initially, VEA found limited applications in liver transplantation and cardiac surgeries.


Dr. Volod

The conventional plasma-based coagulation testing proved challenging for managing patients in various critical clinical scenarios, such as trauma, intraoperative obstetric care, and intensive care. The COVID-19 pandemic dramatically increased the demand for promptly assessing the hemostasis of COVID-19 patients and effectively addressing their coagulation-related complications.

Recent advancements in next-generation VEAs like ROTEM Sigma, TEG 6s, and Quantra have made it possible to perform these tests at the patient's bedside, leading to a broader range of clinical applications. This development has attracted considerable interest from clinicians, laboratory professionals, and hospitals alike.

What prompted you to put this guide together, who is it written for, and is there any similar book on the market?

The project got its start during my tenure on the CAP Hemostasis and Thrombosis Committee during discussions on the rising interest in VEA and the lack of a textbook. I was entrusted to lead the project by the then committee chair and vice chair, Drs. Dong Chen and Andrew Goodwin.

My fascination with VEA traces back to my residency, where I completed an elective rotation in coagulation at the Royal Free Hospital in London, renowned as one of Europe's largest hemophilia centers. It was during this period that I was first introduced to VEA, specifically the TEG. Subsequently, I invested a substantial amount of time and effort over the course of 20 years to acquire an in-depth understanding of various VEAs and their applications.

The primary objective of this book is to offer a thorough yet succinct manual to individuals engaged in viscoelastic testing. It covers aspects such as comprehending hemostasis, the practical application, and the interpretation of VEA across diverse clinical settings. This book caters to a broad audience, including pathologists, clinicians, laboratory scientists, perfusionists, nurses, as well as trainees who depend on viscoelastic testing for patient care and decision-making.

Many articles have been published detailing VEA and its potential applications. However, to the best of my knowledge, this is the inaugural book of its kind on the subject.

The book's first section is an overview of hemostasis physiology, conventional assays, and therapeutic agents, and the second section is devoted to the various FDA-approved viscoelastic assays. Tell us about the third section consisting of case studies and the fourth section on clinical applications.

The book's third section is dedicated to case studies, encompassing various hemostatic disorders in which viscoelastic assays prove valuable. These case studies encompass the clinical histories of real patients, results from traditional and viscoelastic assays, as well as in-depth discussions and reviews of relevant literature. The structured case-oriented format enables authors to explore real-life scenarios and provide accurate diagnoses and interpretations of VEA in correlation with conventional coagulation tests. Each case incorporates pertinent research articles, guidelines, and expert insights, ensuring a comprehensive discussion of the most current evidence-based practices.

In the concluding portion of the book, readers will find an up-to-date exploration of the clinical applications of viscoelastic assays across multiple domains, encompassing areas such as pregnancy, trauma, cardiac surgery, liver transplantation, and neonatal care. Chapters that are dedicated to pregnancy, cardiac surgery, and liver transplantation are supplemented with relevant case studies. Furthermore, where available, transfusion algorithms based on viscoelastic assays are incorporated. Literature on the use of VEA in neonatal care is limited. Dr. Jun Teruya and his coauthors not only address the clinical application of VEA in neonatal cases but also provide valuable information regarding reference ranges in neonatal patients, addressing one of the most common questions I encounter.

What can you tell us about the more than 20 contributors to the book?

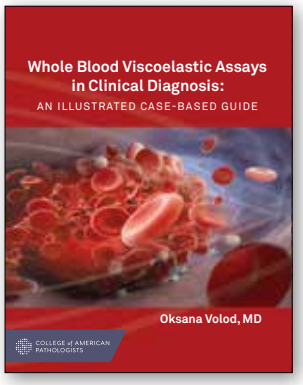
The book owes its existence to an exceptional team of authors with expertise in hemostatic disorders and VEAs, including current and former members of the CAP Hemostasis and Thrombosis Committee (Drs. Chen, Goodwin, and Teruya, and Drs. Huy Pham, James Isom, David Unold, Neil Harris, John Olson, Kristi Smock, Karen Moser, Geoffrey Wool, Mandy VanSandt) and Quality Practices Committee (Dr. Paul Lindholm). Several other pathologists, handpicked by them as coauthors (Drs. Lance Williams, Christina Barriteau, Sumire Kitahara, Erica Swenson, Rasleen Saluja, Amir Navaei, and Amit Gokhale), played a crucial role.

To address the CLIA regulatory requirements for VEA validation, I extended an invitation to Anna Hamilton, our former laboratory quality assurance manager.

Lastly, Dr. Julie Wegner, a recognized expert in the field with extensive experience in TEG and extracorporeal technology, made significant contributions to two chapters of the book.

What is most important for the reader to learn from and take away from your book?

It is my hope that readers will perceive this book as a comprehensive yet concise tool that allows them to refresh their knowledge of hemostasis, understand the FDA-approved and off-label clinical applications of their chosen VEA, and learn how to interpret different VEA results within the context of a patient's medical history. □



Here, from Whole Blood Viscoelastic Assays in Clinical Diagnosis: An Illustrated Case-Based Guide, *is part of chapter 12 on viscoelastic testing and bleeding disorders, by Kristi Smock, MD, and Oksana Volod, MD. The third of the three cases in chapter 12 has been excluded for space reasons, as have the chapter's references and summary.*

To order (PUB231), call 800-323-4040 option 1 ($96 for CAP members, $120 for others; ebook: $86). If you are interested in writing a book, contact Katy Meyer at kmeyer@cap.org.

Viscoelastic Testing and Bleeding Disorders

Introduction

Hemostasis is a complex system that relies on platelet number, function, and platelet–vWF interactions (primary hemostasis); enzymatic pathways of coagulation factors leading to fibrin formation (secondary hemostasis); and fibrin stabilization via cross-linking. In addition, hemostasis is regulated by the system of fibrinolysis that degrades fibrin to fibrin breakdown products. In vivo, the hemostatic system is activated by TF (tissue factor) exposed through tissue injury, and coagulation factors are enzymatically converted to their active forms on the phospholipid surface of activated platelets. The tests most commonly used to assess the hemostatic system include platelet count; platelet function tests; vWF antigen and activity; fibrinogen activity; and clotting times, such as the PT, which measures the extrinsic and common pathways of coagulation, and aPTT, which measures the intrinsic and common pathways of coagulation. D-dimer is also an essential test that measures breakdown products of cross-linked fibrin, and elevations indicate that there is both fibrin formation and fibrinolysis. The commonly used coagulation tests are discussed in more detail in Chapters 1, 13 (Fibrinolysis), and 15 (Platelet Mapping and Other Platelet Function Assays).

Bleeding disorders can be inherited or acquired, and these are differentiated on the basis of clinical bleeding history, medical and medication history, and close correlation with patterns of test results. In general, inherited disorders affect a single hemostatic component, whereas acquired disorders are often more complex, affecting multiple com-

ponents. **Table 12-1** summarizes bleeding disorders along with laboratory tests used in their diagnosis.

Table 12-1. Bleeding Disorders and Laboratory Tests Used in Their Diagnosis.[a]

Disorder	Laboratory Testing
Disorders of Primary Hemostasis	
von Willebrand disease	vWF antigen vWF activity vWF activity/antigen ratio vWF multimers
Platelet function defects	Platelet count (many disorders have a normal platelet count) Platelet aggregometry PFA-100 TEG-platelet mapping Other platelet function tests
Disorders of Secondary Hemostasis	PT aPTT PT and aPTT mixing studies Fibrinogen activity Factor assays Bethesda assays Assays for specific anticoagulant medications
Disorders of Fibrinolysis	Fibrinogen activity D-dimer or other fibrin degradation products Assays of specific fibrinolytic factors Euglobulin clot lysis time
Complex Coagulopathies	Tests for disorders of secondary hemostasis (as above) Platelet count Liver function tests D-dimer

[a]vWF, von Willebrand factor; PT, prothrombin time; aPTT, activated partial thromboplastin time.

Disorders of Primary Hemostasis

von Willebrand Disease. vWF is a multimeric protein that plays an important role in platelet adhesion by serving as a bridge between exposed collagen at the site of an injury and the platelet GPIb/IX/V complex (GPIb). Normal platelet adhesion requires an adequate amount of vWF, presence of the most functional high-molecular-weight forms (larger multimers), and normal interactions with the vWF ligands collagen and platelet GPIb. vWF is also a carrier for coagulation factor VIII. vWD is characterized by a platelet-type bleeding pattern (mucocutaneous bleeding) that encompasses both quantitative (types 1 and 3) and qualitative (type 2 subtypes) abnormalities of vWF. First-line vWD testing panels typically include vWF antigen and activity measurements, factor VIII activity, determination of the activity-to-antigen ratio (which helps to differentiate quantitative from qualitative subtypes), and evaluation of multimeric distribution (because certain type 2 variants demonstrate multimeric abnormalities). Platelet function tests, such as the PFA-100, are often normal in mild vWD but may be abnormal in moderate or severe vWD or in certain vWD subtypes, depending on the methodology used.

Platelet Function Defects. Normal platelet function requires platelet adhesion to an injury site, release of intracellular granules (storage pool), aggregation (through GPIIb/IIIa and fibrinogen), and providing an activated phospholipid surface for activated coagulation factor complexes to generate fibrin. Platelet function is classically assessed by platelet aggregometry LTA, but there are a number of less-labor-intensive methods, such as PFA-100, that are in common use. The classic platelet functional defects are due to inherited absence (or, rarely, qualitative dysfunction) of major platelet surface glycoproteins such as GPIb (Bernard-Soulier disease) or GPIIb/IIIa (Glanzmann thrombasthenia), which result in severe defects of platelet adhesion or aggregation, respectively. Platelet storage pool disorders and release defects are due to absence of platelet granules or abnormal mechanisms for granule release. Inherited aspirin-like defects can lead to impaired platelet thromboxane generation.

Acquired platelet dysfunction can be due to uremia, medications, or certain foods and supplements. Antiplatelet medications are used to decrease the risk of arterial thrombotic events and cause platelet dysfunction by blocking platelet surface receptors (such as the ADP receptor antagonist clopidogrel or the GPIIb/IIIa inhibitor abciximab) or by interfering with thromboxane generation (such as with aspirin blockade of the COX-1 enzyme). Although most platelet function studies are ordered to assess for inherited platelet dysfunction, evaluation to assess the effects of antiplatelet medications may be performed in some settings.

Platelet function testing methodologies are described in detail in Chapter 15. TPM methodology is described in Chapter 7; current use of TPM in the evaluation of platelet dysfunction is discussed in Chapter 15.

Disorders of Secondary Hemostasis

Secondary hemostasis is initiated by tissue injury, followed by conversion of coagulation factors to their activated enzymatic or cofactor forms, resulting in thrombin generation and fibrin formation. In vivo, the initiation phase involves the extrinsic (TF and factor VII) and common (factors X, V, and II [prothrombin] and fibrinogen) pathways of coagulation (mimicked by the PT test); the propagation phase involves the intrinsic (factors VIII, IX, and XI) and common pathways of coagulation (mimicked by the aPTT test). Contact factors XII, prekallikrein, and high-molecular-weight kininogen are used to initiate the aPTT clotting time but are not necessary for normal in vivo hemostasis. Fibrin is stabilized by factor XIII cross-linking and eventually is broken down during fibrinolysis.

Aside from the contact factors, significant deficiency or dysfunction of coagulation factors manifests as a bleeding disorder. Inherited coagulation factor defects tend to involve a single coagulation factor, such as the factor VIII deficiency in hemophilia A. The most common acquired factor deficiencies seen in clinical practice are multiple deficiencies (liver disease, disseminated intravascular coagulation, etc.) and will be discussed below. However, acquired antibodies/inhibitors of single coagulation factors can also rarely occur (such as an acquired factor VIII inhibitor) and can be titered using Bethesda assays (BAs). Coagulation factor abnormalities lead to prolongations of clotting times, such as PT and/or aPTT, depending on which factor(s) are involved; patterns of these clotting times can be used to select factor activity assays for more definitive information. PT and aPTT mixing studies can be used to determine if a prolongation is due to a factor deficiency or demonstrates an inhibitor pattern (caused by coagulation factor inhibitors, lupus anticoagulants, and certain anticoagulant medications). Because PT and aPTT can be insensitive to mild or moderate fibrinogen deficiency or dysfunction (dysfibrinogenemia), fibrinogen activity studies should always be performed as part of a bleeding disorders assessment. The TT test is very sensitive to fibrinogen deficiency or dysfunction but is also sensitive to anticoagulants such as heparin or direct thrombin inhibitors. Factor XIII deficiency/dysfunction causes weak clots that lyse more readily; however, the PT and aPTT are normal in this disorder, and specific factor XIII testing must be used for diagnosis. The historic qualitative clot lysis test is sensitive to only the most severe factor XIII deficiency (less than one to three percent activity), and quantitative activity tests have become available more recently. The mechanism of anticoagulant drugs is to create coagulation factor deficiencies or to inhibit activated coagulation factors, impeding thrombin and fibrin formation. Anticoagulants will be discussed further in Chapter 14.

Disorders of Fibrinolysis

Fibrinolysis is the process of clot dissolution mediated by the key enzyme plasmin. Derangements of the fibrinolytic pathway can lead to a bleeding disorder (discussed further in Chapter 13).

Multiple or Complex Coagulopathies

Many acquired coagulopathies involve multiple or complex defects. For example, nutritional vitamin K deficiency or warfarin therapy results in deficiencies of the vitamin K-dependent coagulation factors (II, VII, IX, and X), and severe abnormalities of liver synthetic function result in deficiencies of all coagulation factors except VIII, which is not produced by the liver parenchyma. In contrast, disseminated intravascular coagulation (DIC) is defined by systemic activation of the coagulation system, with consumption of all procoagulant and anticoagulant factors and platelets, activation of the fibrinolytic system, and generation of high levels of fibrin degradation products such as D-dimer. Because of this complexity, DIC has clinical manifestations of both bleeding and clotting. When coagulation factor deficiencies are multiple, clotting times such as the PT and aPTT are affected differently from situations with singular deficiencies, and they display prolongation when factor levels are at the low end of normal or mildly low. Factor assay patterns characteristic of multiple or complex coagulopathies are shown in **Table 12-2**.

Table 12-2. Factor Assay Patterns Characteristic of Multiple or Complex Coagulopathies.[a]

	Vitamin K/Warfarin	Liver Disease	DIC
Fibrinogen (factor I)	N	Varies	↓
Factor II	↓	↓	↓
Factor V	N	↓	↓
Factor VII	↓	↓	↓
Factor VIII	N	↑	↓
Factor IX	↓	↓	↓
Factor X	↓	↓	↓
Factor XI	N	↓	↓
Factor XII	N	↓	↓

[a]DIC, disseminated intravascular coagulation; N, normal; ↓, decreased; ↑, increased.

Conventional laboratory assays (PT and aPTT) for a bleeding patient may reflect poor in vivo hemostasis, may overlook platelet dysfunction or fibrinolysis, or may be spuriously abnormal because of the presence of lupus anticoagulant. VET, on another hand, has capability to assess various steps of clot formation and fibrinolysis in one assay and is not affected by lupus anticoagulant. The following cases will illustrate the potential role and limitations of VET in bleeding disorders assessment.

Case 4: Preoperative Workup of Abnormal PFA-100

History. This case involves a 60-year-old man with spontaneous subdural hematoma while taking a nonsteroidal anti-inflammatory drug for one month. He has a history of childhood epistaxis that improved with age. There is no other personal or family history of bleeding. Several surgeries in the past were without complications.

Table 12-3. Case 4: Results of Conventional Laboratory Assays.

Laboratory Assay	Patient's Results	Reference Interval
PT (seconds)	14.1	11.0–14.4
aPTT (seconds)	28	22–37
Platelets (K/µL)	235	150–450

Laboratory Tests. Results of conventional laboratory tests are shown in **Table 12-3**.

PFA-100: Normal and CADP Abnormal.

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Figure 12-1. TEG 5000 graph from Case 4, demonstrating an overall normocoagulable profile with normal clotting time (R), α angle, and maximal amplitude (MA). Reference tracings are superimposed in the background (blue dashed line).

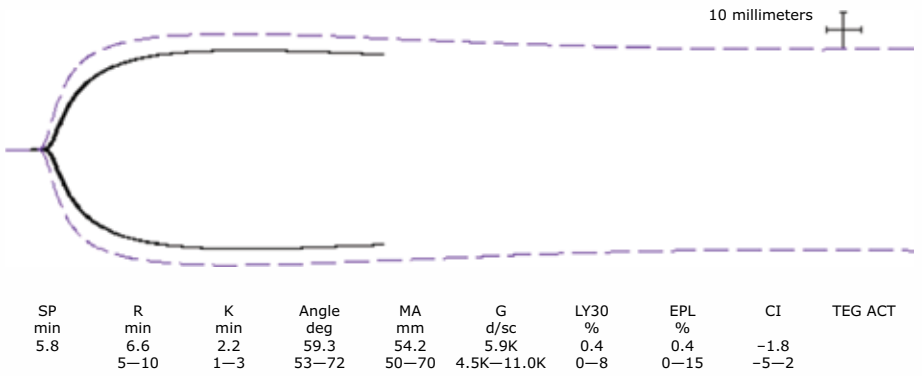
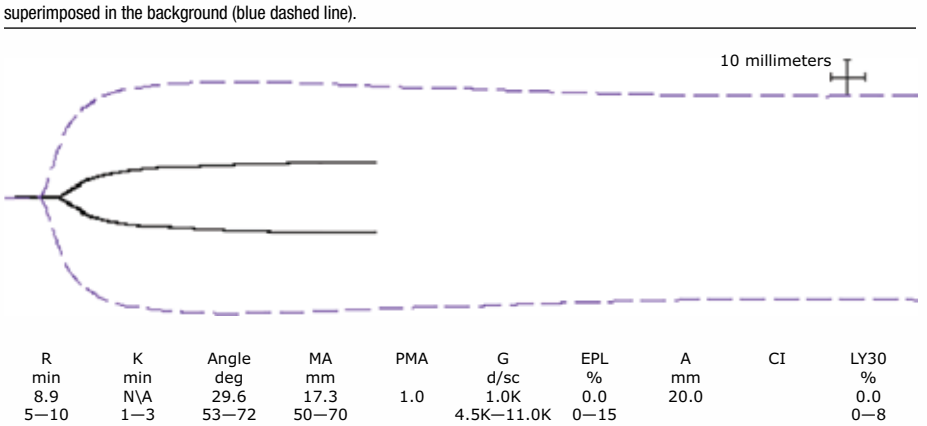


Figure 12-2. TEG 5000 graph from Case 5, demonstrating a hypocoagulable profile, which is mainly due to the decreased platelets/fibrinogen contribution to the clot stability. This is evidenced by low α angle and maximal amplitude (MA). Reference tracings are superimposed in the background (blue dashed line).



Viscoelastic assays

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TEG 5000 Interpretation. In **Figure 12-1**, the data show a normal clotting time (R = 6.6 minutes), indicating normal rate of thrombin generation. The α angle and MA are also normal, indicating adequate fibrinogen contribution to the clot stability, as well as normal platelet function potential. TPM (not shown) shows no significant platelet function inhibition.

A vWD panel that was performed as part of the abnormal PFA-100 showed decreased vWF activity, suggestive of type 1 vWD (**Table 12-4**).

Table 12-4. Case 4: Results of von Willebrand Disease (vWD) Panel.

Test	Patient's Results	Reference Interval
Fibrinogen (mg/dL)	337	200–400
von Willebrand factor antigen (%)	24	50–160
von Willebrand factor activity (%)	18	>39
Factor VIII:C (%)	66	50–150
Multimers	Normal	Normal

Discussion of Case 4. vWF release and activity is influenced by the high shear rate stress that is found in small arteries, stenotic heart valves, and ventricular assist devices. Standard VET measures hemostatic potential of the blood under low shear rate forces, simulating venous flow, and are not sensitive to detect vWF dysfunction, as is shown in Case 4.

TF-activated TEG has been shown to identify impaired clot formation in patients with vWF less than 30 percent; however, this assay is not a standard kaolin-activated TEG assay. Modified ROTEM using ristocetin activation has also been found to be useful in the diagnosis of vWD.

Case 5: Abnormal PFA-100 Assessment in a Patient with Lifelong Bleeding Diathesis

History. This case involves a 44-year-old Asian woman with long-standing history of gum bleeding (starting at two to three years of age), epistaxis (three to four days' duration), and menorrhagia (20 to 30 days' duration) since her teenage years. During Caesarean section, she developed massive hemorrhage requiring more than 10 units of packed RBCs. There is a family history of similar bleeding disorders in two of five brothers. vWD workup (performed at an outside institution) was within normal ranges.

Laboratory Tests. Results of conventional laboratory tests are shown in **Table 12-5**.

Table 12-5. Case 5: Results of Conventional Laboratory Assays.[a]

Laboratory Assay	Patient's Results	Reference Interval
PT (seconds)	12.8	11.0–14.4
aPTT (seconds)	32	22–37
Platelets (K/μL)	150	150–450
Fibrinogen (mg/dL)	384	200–400
Hemoglobin (g/dL)	10.6	12.0–15.5

[a]PT, prothrombin time; aPTT, activated partial thromboplastin time.

PFA-100: CEPI Abnormal and CADP Abnormal. Blood smear review showed morphologically normal platelets.

TEG 5000 Interpretation. In **Figure 12-2**, the data show a normal clotting time (R = 8.9 minutes), indicating a normal rate of thrombin generation. The α angle and MA are significantly decreased, indicating decreased fibrinogen/platelets contribution to the clot stability. With normal fibrinogen function, the defect is platelet related. TPM (not shown) shows significant platelet function inhibition. There is no fibrinolysis on the basis of the LY30 parameter, which measures clot lysis after 30 minutes of maximum clot strength.

Platelet Aggregometry. Platelet aggregometry, which is the gold standard assay for platelet function, was also performed. It showed markedly abnormal platelet aggregation with all platelet agonists (**Figure 12-3**).

In summary, this patient's abnormal PFA-100 and lifelong personal and family history of bleeding diathesis are suggestive of the inherited platelet disorder Glanzmann thrombasthenia.

Discussion of Case 5. In TEG, clotting is initiated by the addition of kaolin and calcium, which generates a strong thrombin response that maximally activates all platelets through the protease-activated receptor-1 receptor, which is unaffected by aspirin, nonsteroidal anti-inflammatory medications, and the $P2Y_{12}$ antagonists. TPM allows the assessment of non-thrombin-based platelet activation/inhibition (refer to Chapters 7 and 15 for more information).

TEG MA is a direct measurement of the polymerizing fibrin and activated platelet/fibrinogen interactions via GPIIb/IIIa receptors and represents the maximal strength of the clot. Therefore, thrombocytopenia, drugs (for example, tirofiban, abciximab), or inherited platelet disorders (for example, Glanzmann thrombasthenia) affecting GPIIb/IIIa receptors will affect (reduce) the TEG MA parameter, as shown in Case 5. The ROTEM MCF parameter is analogous and will be affected. Fibrinogen facilitates platelet aggregation via GPIIb/IIIa receptors, but in Glanzmann thrombasthenia, the platelet/fibrinogen interaction will be decreased, and this will affect not only MA (TEG) or MCF (ROTEM) but also K, α angle (TEG), and clot formation time (ROTEM). Defects in another major platelet surface glycoprotein, GPIb (Bernard-Soulier disease), leads to a defect in platelet adhesion to the endothelium and is also characterized by thrombocytopenia and large platelets. VET parameters assessing platelet contribution to clot stability (for example, MA, MCF) via thrombin activation do not appear to be significantly affected. However, when the effect of thrombin is removed, as occurs in TPM, clot formation/stability when blood is activated with an ADP agonist is significantly reduced.

Glanzmann thrombasthenia is a very rare disorder that is inherited in an autosomal recessive pattern. The disease occurs with greater frequency in populations in which intermarriage within a group (consanguinity) is more prevalent. Acquired thrombasthenia has been reported in patients with acute promyelocytic leukemia—chromosome translocation t(15;17)—when the breakpoint region on chromosome 17 occurs at 17q21, which is the location of the genes for αIIb and β3. Autoantibodies with specificity for platelet GPIIb/IIIa have also been reported. The diagnosis is suspected in patients with mucocutaneous bleeding, absent clot retraction, abnormal PFA-100, absent platelet aggregation in response to all agonists (except ristocetin), and a normal platelet count and morphology. The diagnosis is confirmed by flow cytometry demonstrating platelet αIIbβ3 receptor deficiency. Flow cytometry may not reveal the variant with receptor dysfunction. Therapeutic management for inherited disease includes administration of desmopressin (brand name: DDAVP) and platelet transfusion. Platelet aggregometry may only be available in specialized coagulation laboratories, whereas VET is more widely available and may be useful in providing evidence of the existence of a bleeding disorder in symptomatic patients. Measurements of platelet counts and platelet function with PFA-100 or platelet aggregometry do not necessarily correlate with generation of stable thrombi. These patients may require frequent platelet transfusions and may develop platelet refractoriness to platelet transfusion. VET may provide an additional benefit by guiding treatment of such patients undergoing surgical procedures. □

Figure 12-3. Platelet aggregometry shows markedly decreased aggregation with adenosine diphosphate (1), epinephrine (2), collagen (3), and arachidonic acid (4).