

overview [phlebotomy | coagulation and hematology]

The Evolution of Evacuated Blood Collection Tubes

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- ▶ The introduction of evacuated tubes greatly enhanced the precision and accuracy of test results by reducing errors in collection, (eg, blood-to-additive ratios or contamination).
- ▶ This article reviews the history of evacuated tubes, the regulations and manufacturing of evacuated tubes, the additives used in evacuated tubes, and some environmental factors influencing product performance.

“A laboratory test is no better than the specimen, and the specimen no better than the manner in which it was collected.” So stated the advertising language of BD (Becton Dickinson) to promote the first evacuated blood collection tubes, back in the late 1940s and early 1950s.¹ This technology for blood collection, patented in 1949, is substantially similar to the technology pervasive in clinical practice today.

Consider what it was like to draw blood without an evacuated tube system. Even before collecting blood, the laboratory had to prepare solutions for the additive tubes (eg, EDTA, citrate) and dispense them into test tubes for blood anticoagulation. Then, to identify the proper draw volume, the laboratory had to etch lines in the borosilicate glass tubes. The phlebotomist collected blood specimens with needles and glass syringes. For patients who required many tests, the phlebotomist might have to stick the patient multiple times, at least once for chemistry, once for hematology, and once for coagulation.

After collection, the phlebotomist would transfer the blood into a series of test tubes. They sealed the tubes with black rubber stoppers for transportation of the specimens to the laboratory. For

electrolyte measurements, they added mineral oil to the tubes to prevent loss of CO₂. For serum specimens, the technologist would use wooden applicator sticks to loosen the clot from the tube walls.

Before drawing blood from the next patient, the laboratory would wash the syringes and tubes. This required many rinse cycles to remove all of the soap residue. Needles were resterilized and occasionally resharpener using a grinding wheel.

The shortcomings of these techniques are numerous. First, the patient is subjected to the pain of multiple-needle entries to the vein. Secondly, the possibilities for errors to occur during the collection and transfer process and the safety risks are apparent. Also, time is consumed with the multiple punctures and transfers.

History of Evacuated Tubes

The introduction of evacuated blood collection systems provided greater safety, while offering ease-of-use, speed, and accuracy in blood-to-additive ratios. Many advancements in blood collection techniques and devices have been made in recent years.² However, the technique of blood collection with the first evacuated tubes was not quite the same as the process used today. During blood collection with evacuated tubes, as one end of the needle enters the patient's vein, the other end can penetrate through the rubber stopper as the tube is pushed into the open end of the holder. The vacuum enables the tube to fill with the appropriate volume of blood. Additional tubes may be inserted into the holders after completion of the previous draw, when multiple specimens are required.

The first evacuated tube patent, Evacutainer, was invented by Joseph Kleiner and assigned to BD in 1949 [F1 and I1].³

Prior to the issuance of the patent, Kleiner approached BD with the Evacutainer. BD subsequently hired Kleiner as a consultant for the product and changed the name of his tube to Vacutainer™. Shortly thereafter, it became one of the company's largest selling items.

BD Vacutainer™ tubes were packaged and shipped in vacuum tins similar to coffee cans [I2]. This was a breakthrough at the time because previously, a heavy clamp was used to prevent the stoppers from popping off during autoclaving. Initially, BD made only 1 kind of Vacutainer™ tube, but now it makes hundreds of styles and sizes. The current evacuated tube system utilizes color-coded stoppered tubes containing the vacuum and a holder that supports a double-ended needle. The color-coded closures differentiate tube types.

BD was the only evacuated tube manufacturer in the United States until the early 1970s when other manufacturers entered the market. F2 illustrates a historical time line for FDA clearance of evacuated tubes. Contact information for tubes currently sold in the United States is shown in T1.

Today, there are regulatory agencies and guidelines that ensure the consistency in the design and manufacture of blood collection systems [eg, Food and Drug Administration (FDA); International Standardization Organization (ISO); and National Committee for Clinical Laboratory Standards (NCCLS)].⁴⁻⁶ Major regulatory developments in the evolution of evacuated tubes are also depicted in F2. Federal requirements governing investigations involving medical devices were enacted as part of the Medical Device Amendment (1976) and the Safe Medical Devices Act (1990).⁷ These amendments to the Federal Food, Drug and Cosmetic

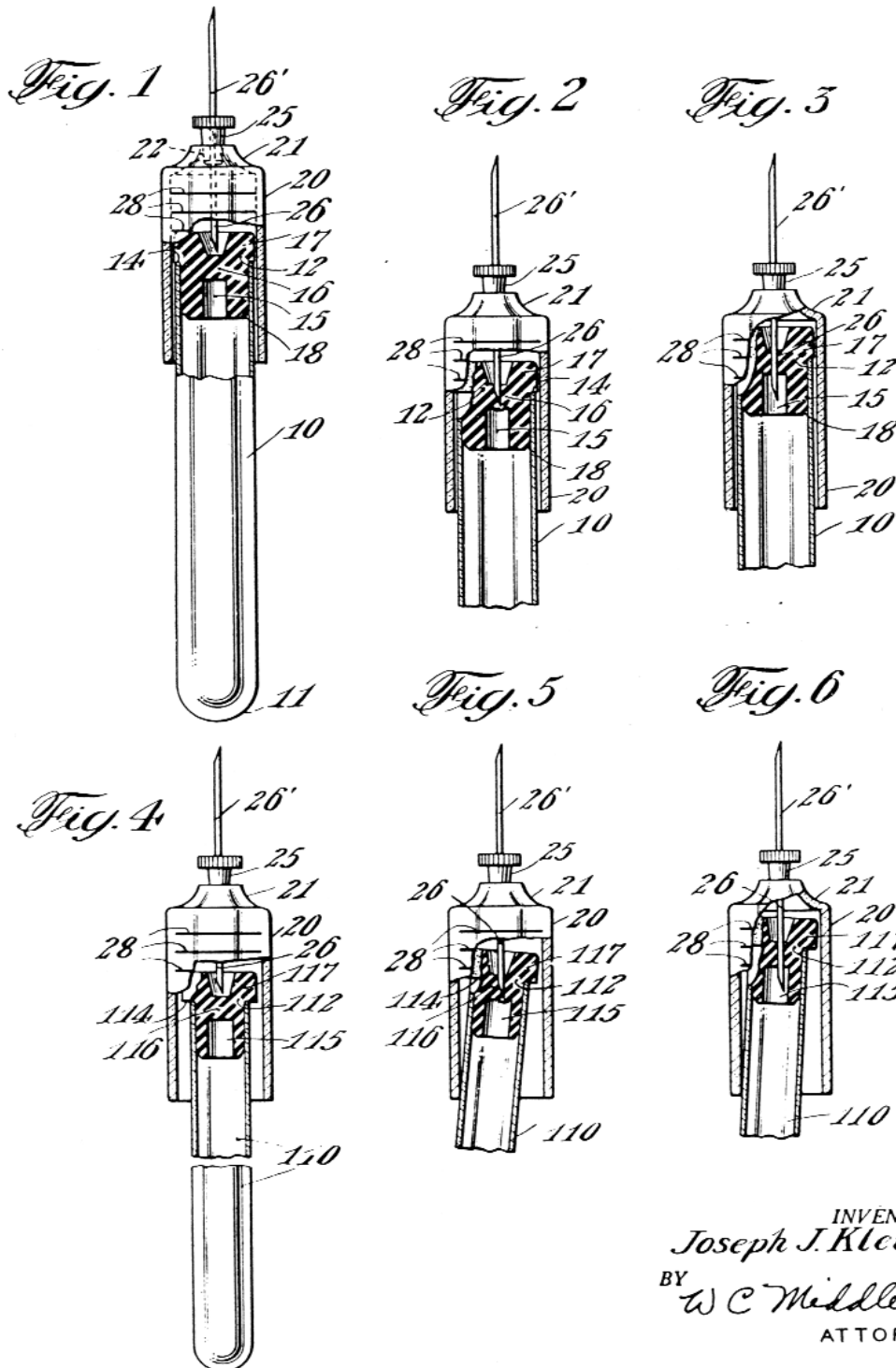
Feb. 1, 1949.

J. J. KLEINER

2,460,641

BLOOD COLLECTING APPARATUS

Filed Aug. 14, 1945

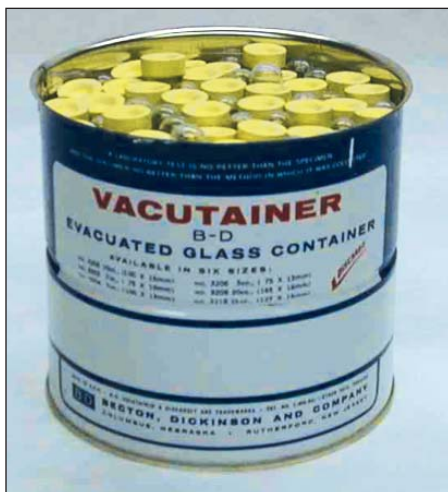


INVENTOR.
Joseph J. Kleiner
BY *W. C. Middleton*
ATTORNEY

[F1] Copy of the original patent for the BD Vacutainer™ Blood Collection System.³ (Reprinted with permission.)



[11] Original Evacutainer® tubes and packaging.



[12] Packaging of Vacutainer™ tubes in vacuum cans.

mark. Furthermore, all class I and II medical devices sold in the United States must receive clearance from the FDA and Center for Device and Radiologic Health (CDRH) prior to sale. Included in the FDA's review of the 510k (premarket notification) are the physical description of the product and contents, as well as product performance for safety and effectiveness. General manufacturing practices are described below.

Glass evacuated blood collection tubes can be made from glass canes cut to predetermined lengths and fired at 1 end to close the bottom. Plastic blood collection tubes may be manufactured by an injection-molding process.¹¹ A mold is made to the specific size of tube desired. Typically, in the molding process, a hot, molten material is injected into a cold mold for the tube. After the tube material cools and solidifies, the mold is opened, and the tube is ejected.

Once the tube is formed, additives may be topically applied and dispersed along the inner wall of the tube.¹² Most of these additives are considered to be “dry.” Tubes are spray coated with additive formulations onto the inner wall using a series of nozzles. Dispensing is achieved by either pressure activation or volume displacement. The coating is dried by forced air or vacuum. Alternatively, additives that are dispensed into the tube as a fluid and remain as a liquid are considered “wet.” A gel barrier may also be dispensed into the formed tube for gel separator tubes.

After any additive or gel is inserted, the tubes are then evacuated and stoppered. An evacuating-closure device evacuates the interior of the tube and applies a stopper to the opening of the tube.¹² The tubes are subsequently labeled appropriately. In the early days, evacuated tubes were hand assembled and not sterilized, but now manufacturers in the United States run automated machine lines and sterilize their tubes. Sterilization is typically accomplished by irradiation after evacuation and is now only rarely achieved through autoclaving.¹³ After sterilization the tubes are wrapped and boxed for shipping.

on the market prior to 1976 were grandfathered from the premarket notification (to the FDA) requirement. The Needle Stick Safety and Prevention Act revises and builds upon the Bloodborne Pathogen Directive promulgated by the federal Occupational Safety and Health Administration (OSHA).¹⁰ Provisions of the new law require changes to an institution's current exposure control plan to include ‘safety-engineered’ products for blood collection. The definition of safety-engineered medical devices includes plastic evacuated tubes with shielded caps.

Manufacturing Evacuated Tubes

At least 2 standards organizations, NCCLS and ISO, have promulgated standards for the manufacturer of evacuated tubes.^{5,6} These standards define the tube dimensions for compatibility with centrifuges and automated instruments, draw and fill accuracy, types of additives and additive tolerances, sterility, and labeling criteria. Manufacturers are encouraged to follow these guidelines to obtain CE

Act define the regulatory framework for medical device development, testing, approval, and marketing. Additionally, the Federal Quality System Regulation (QSR) and ISO define quality system requirements for the manufacture of medical devices.^{8,9} Any class I or II products

Materials in Evacuated Tubes

Tubes

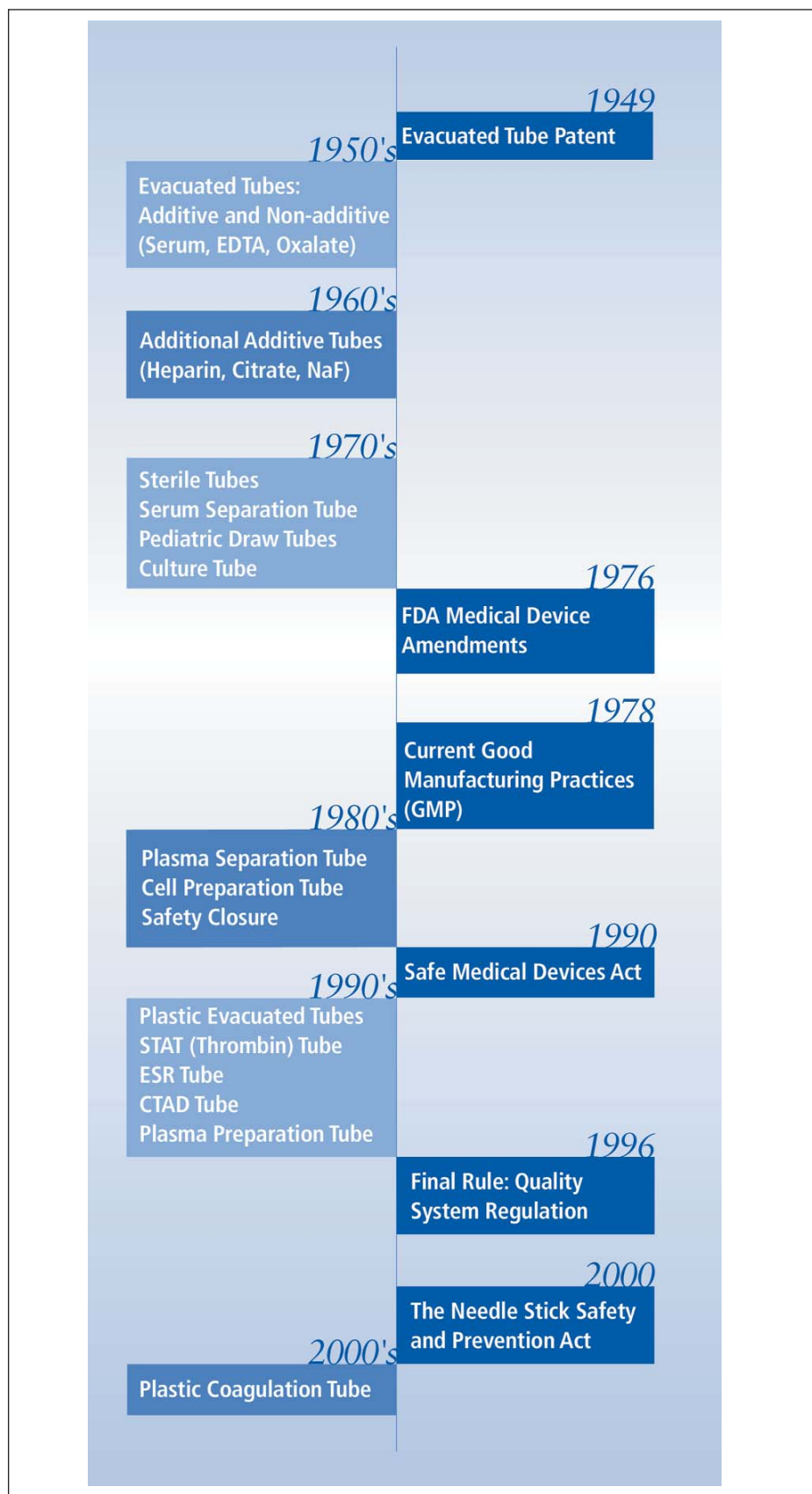
Today, evacuated tubes are available in glass or plastic, although a trend toward plastic is occurring due to recent safety legislation.¹⁰ Most often, the plastic material is polyethylene terephthalate (PET). Glass and PET are viable materials for the tubes because they are clear, they can hold a vacuum for an extended period, and they have the toughness to withstand centrifugation.¹⁴

There are benefits to using tubes made from either material. The plastic tubes are less likely to break and with the growing concerns about the spread of bloodborne pathogens, this is an important safety feature. Furthermore, the plastic tubes weigh less than glass tubes, reducing costs associated with transport and disposal. However, glass tubes provide a greater barrier to air and water vapor transfer than PET. In later sections, we discuss how this difference in barrier properties affects tube expiration dates, shelf-life properties, and preanalytical variables.

Additives

Although there are evacuated glass blood collection tubes without additives used to yield serum, all other evacuated tubes contain at least some type of additive. Many of these additives are the same as those used in transfer tubes before evacuated tubes were introduced. The additives range from those that promote faster clotting of the blood, to those that enable anticoagulation, and to those that preserve or stabilize certain analytes or cells. The inclusion of additives at the proper concentration in evacuated tubes greatly enhances the accuracy and consistency of test results and facilitates faster turnaround times in the laboratory.

Additives may exist as either dry or liquid (“wet”) in evacuated tubes depending on whether the tube is glass or PET, and depending on the stability of the solution. The NCCLS and ISO define the concentrations of these additives dispensed into tubes per milliliter of blood. Although the additives themselves may be stable in plastic evacuated tubes,



[F2] Historical time line of evacuated tubes²¹

Web Sites for Major Evacuated Tube Manufacturers in the US

T1

Name Brand Names

Becton Dickinson & Co. Franklin Lakes, NJ 800-631-0174 www.bd.com/vacutainer	BD Vacutainer™
Greiner Mediatech Baltimore, MD 888-286-3883 www.greiner-lab.com	VACUETTE®
Sherwood Davis & Geck St. Louis, MO 800-527-1973 www.kendallhq.com	MONOJECT®
Sarstedt, Inc.* Newton, NC 800-257-5101 www.sarstedt.com	Monovette®

*Sarstedt does not manufacture evacuated tubes, although these products can be used in aspiration mode to simulate evacuation.

the water in which they are dissolved can diffuse out of a plastic tube over time. If water vapor escapes from the tube, the dilution provided by the remaining water in the tube is reduced, potentially affecting results. Using the products within the manufacturer's stated shelf life ensures minimal water loss and the correct blood-to-additive ratio.

Inorganic Additives

There are several different types of inorganic additives in blood collection tubes. These include those that are completely inorganic in composition, (eg, silica and sodium fluoride), and those that are alkaline metal salts of organic acids, [(eg, disodium ethylenediaminetetraacetic acid (Na₂EDTA), K₃ or K₂EDTA, trisodium citrate, and potassium oxalate)]. Most dry additives tend to not be a limiting factor in determining the shelf life of the evacuated tube.

For hematology applications, EDTA is available in 3 forms, including dry additives (K₂EDTA or Na₂EDTA) and a liquid additive (K₃EDTA). EDTA is combined with a metal cation to enhance its solubility and maintain pH. K₂EDTA is slightly more soluble than Na₂EDTA and is the anticoagulant recommended by the

International Council for Standards in Hematology.¹⁵ EDTA is an efficient anti-coagulant which does not affect cell counts and minimally affects cell size. EDTA prevents clotting by chelating calcium, an important cofactor in coagulation reactions. The amount of EDTA per milliliter of blood is essentially the same for all 3 forms of EDTA (1.5-2.2 mg/mL).⁶ However, slight differences in hemoglobin may be observed between K₂EDTA and K₃EDTA due to dilutional effects from K₃EDTA.¹⁵

For coagulation testing, only liquid additives are currently available. This is to preserve the 9:1 ratio (blood:citrate) recommended for coagulation testing.¹⁶ To maintain this ratio, coagulation tubes are typically available in glass (to reduce water loss). Evacuated plastic coagulation tubes consisting solely of PET have special packaging to prevent water vapor from escaping over the shelf life of the tube. Also available are plastic evacuated tubes containing liquid additives with a polypropylene insert tube within the PET outer tube. In such configurations, the inner polypropylene tube prevents the loss of water vapor, while the outer PET tube preserves the vacuum.

Trisodium citrate (Na₃C₆H₅O₇·2H₂O), buffered or unbuffered, is the current standard anticoagulant for coagulation testing. Some manufacturers buffer with citric acid to maintain the pH and minimize damage to the glass tube wall. Some manufacturers may also coat the glass tube wall. Before coagulation testing became automated, several citrate concentrations in evacuated tubes were available. Today, it is available as either a 3.2% or 3.8% concentration. Different citrate concentrations can have significant effects on aPTT and PT results so interchanging these within a laboratory is not recommended.¹⁶ The different citrate concentrations affect different patient populations and reagent responsiveness.¹⁷

Biochemical Additives

Biochemical additives or enzymes (ie, heparin or thrombin) are susceptible to instability and/or degradation. Exposure to irradiation during sterilization of tubes and to moisture or light during the

shelf life of the product can limit the stability of biochemical additives. As a result, some of these additives are often only available in glass tubes. When biochemical additives are placed in plastic tubes, they may require special packaging to maintain stability.

Heparinized plasma is commonly used in chemistry in STAT or routine analyses. Heparin is a mucopolysaccharide combined with a cation (Na⁺, Li⁺, NH₄⁺) to enhance its solubility. It anticoagulates blood by inhibiting thrombin and Factor Xa. Evacuated heparin tubes contain 10 to 30 USP units/mL blood.⁶

A special additive mixture that is found only in glass evacuated tubes for coagulation testing is called CTAD (citric acid, theophylline, adenosine, and dipyridamole). The CTAD cocktail minimizes platelet activation after blood collection.¹⁸ This additive is sensitive to light and is currently only available in glass evacuated tubes.

Thrombin tubes are often used for STAT serum testing due to the short clotting time. Thrombin is an enzyme that converts fibrinogen to fibrin. Thrombin tubes are available in either glass or plastic. Plastic thrombin tubes require special packaging to protect against moisture. Thrombin is dispensed into tubes to 2 NIH units/tube.⁶

Gel

Corning Medical's, now Sherwood Medical, Corvac® tube was the first gel tube marketed. BD gel tubes were introduced in 1975, and BD re-introduced with a different gel material in 1976. Terumo followed BD with the AUTOSEP® tube in the early 1980s. The function of the gel is to provide a physical and chemical barrier between the serum or plasma and the cells. The usage of gel barriers has provided a large benefit in collecting, processing, and storage of the specimen in the primary tube.

Separator gels are capable of providing barrier properties because of the way they respond to applied forces. After blood is drawn into the evacuated gel tube, and once centrifugation begins, the g-force applied to the gel causes its viscosity to decrease, enabling it to move or

flow. Materials with these flow characteristics are often called thixotropic. Once centrifugation ceases, the gel becomes an immobile barrier between the supernatant and the cells. The nature of the movement of gels in evacuated blood collection tubes is described elsewhere.¹⁹

When first introduced, separator tubes contained a silicone gel, but these were unstable after sterilization. Gels are generally comprised of more than 1 component. They may consist of a primary organic phase, referred to as a resin, an inorganic powder, and a network stabilizer.²⁰ The inorganic phase is needed to adjust the density of the gel so that it is between the density of the serum or plasma and the cells. To render the organic and inorganic phases compatible, a chemical stabilizer must be added as another component to the gel. Due to the composite nature of gels, the shelf life of gel tubes is finite.

Expiration Dates of Evacuated Tubes

The expiration dates of glass tubes are generally limited by the shelf life of the additives because vacuum and water-vapor losses are minimal over time. As reviewed in the earlier sections, gels and additives sensitive to irradiation and the environment are often the limiting factors in determining expiration dates for glass tubes.

The expiration dates of evacuated plastic tubes are often also limited by the same factors that were noted for glass tubes. In addition, evacuated plastic tubes do sustain a measurable loss of vacuum over time, and some evacuated plastic blood collection tubes may have their expiration dates determined by their ability to assure a known draw volume. Because of the lower water-vapor barrier properties of PET that have already been discussed, the expiration dates of wet additive plastic evacuated tubes are determined by the barrier provided either by the insert tube or by the special packaging. Most evacuated tubes on the market have at least a 12-month shelf life.

Expiration dates are determined through shelf-life testing performed under known environmental conditions. Shelf life of an evacuated tube is defined by the

stability of the additive, as well as vacuum retention. The NCCLS and ISO state that the draw volume of an evacuated tube must be within $\pm 10\%$ of the label draw throughout the shelf life.^{4,5} This limit is set to minimize significant changes in blood-to-additive ratios. If the environmental conditions under which evacuated tubes are stored are not consistent with those recommended by the manufacturer, it is possible that the draw volume of the tubes may be affected. In the next section, we will discuss the potential impact of such environmental factors on the draw volume of evacuated tubes.

Environmental Factors Affecting Evacuated Tubes

It is important to understand that evacuated blood collection tubes are not completely evacuated. In fact, there is a small amount of gas (air) still residing in the tube, at low pressure. The higher the pressure of the gas inside the tube on the date of manufacture, the lower the intended draw volume will be for a tube of a given size. The draw volume specified for a given tube is achieved by manufacturing the tube at a designated evacuation pressure.

The dynamics of blood collection inside the tube are based on the ideal gas law:

$$PV=nRT$$

In the equation, P is the pressure inside the tube, V is the volume that the gas occupies, n is the number of moles of gas inside the tube, R is the universal gas constant, and T is the temperature inside the tube.

According to the equation, if the moles of gas and the temperature do not change, the product of pressure and volume is a constant. Consider now the role of the residual gas during the blood collection process in an evacuated tube. When blood starts filling the tube, the residual gas inside is confined into a decreasing volume, causing the pressure of the gas to increase. When the pressure of this gas reaches ambient pressure, the collection process is completed for that tube.

Note that because there is gas inside the tube on the date of manufacture, environmental conditions could alter the pressure of this gas inside the tube during

storage and impact the resulting draw volume. Therefore, it is important that tubes be stored under recommended conditions.

Temperature

To understand the impact of temperature on draw volume, the ideal gas law is again applied. According to the equation, if tubes are stored at low temperature, the pressure of the gas inside the tube will decrease. This would lead to an increase in draw volume for the evacuated tube. Conversely, higher temperatures could cause reductions in draw volume.

These reductions in draw volume could be exacerbated at higher temperatures in plastic evacuated tubes. This occurs because the permeability of the plastic to gas increases with increasing temperature. Thus, more moles of gas migrate inside the tube resulting in greater rates of draw loss over time. Additionally, PET is less resistant to high temperatures than glass. When plastic tubes are exposed to very high temperatures ($\geq 60^{\circ}\text{C}$), the plastic softens, and under worst-case conditions, may deform.

For plastic tubes that contain a liquid additive, the water-vapor permeability of the tube will increase with temperature. This enables water to escape at a faster rate, potentially altering the blood-to-additive ratio.

Also, it should be noted that the stability of certain other tube additives, for example, biochemicals or even gel, could be negatively impacted by increased temperature in evacuated tubes. As discussed above, gel is a heterogeneous compound that could possibly sustain some degradation when exposed to high temperatures.

Altitude

In situations where blood is drawn at high altitudes ($\geq 5,000$ feet), the draw volume again may potentially be affected. Because the ambient pressure at high altitude is lower than at sea level, the pressure of the residual gas inside the tube will reach this reduced ambient pressure during filling earlier than if the tube were drawn at sea level. Hence, the draw volume will be correspondingly lower. Some manufacturers may compensate for this effect by providing tubes that have been

evacuated to lower pressures for customers drawing blood at high altitude.

Humidity

The impact of storage under different humidity conditions could potentially impact only plastic evacuated tubes, due to the greater permeability of these materials to water vapor relative to glass. Conditions of very high humidity could lead to the migration of water vapor inside a tube that contains a moisture-sensitive material, such as a lyophilized additive. Conditions of very low humidity could hasten the escape of water vapor from a tube containing a wet additive. It is possible that such storage conditions could compromise the accuracy of clinical results.

Light

As previously mentioned, the CTAD additive is photosensitive. Normally, this additive has a slightly yellow appearance that becomes clear when no longer viable. These tubes are generally packaged in small quantities to minimize exposure to light.

Summary

Blood collection and analysis are useful diagnostic tools in the practice of laboratory medicine. The advent of evacuated blood collection tubes significantly improved accuracy and precision, safety, ease-of-use, and speed of the diagnostic process. We have shown the progression of technology and regulatory events that have brought evacuated tubes to their current status.

We have discussed factors that influence evacuated tube performance. Tube material, additive stability, and environmental conditions impact the expiration dates of certain tubes. It is important to store evacuated blood collection tubes under the conditions recommended by the manufacturer to assure an accurate draw volume and clinical results over the shelf life of the product.

1. Becton Dickinson and Company. Joseph Kleiner and the origins of the Vacutainer™. *The Echo*. Becton Dickinson and Company, Franklin Lakes, NJ: 1991 (Spring);11:3-5, 1991(September);11:5-7; 1996(December);16:1
2. Bush VJ, Leonard L and Szamosi DI. Advancements in blood collection devices. *Lab Med*. 1998;29:616-622.

3. Kleiner J. "Blood collecting apparatus", U.S. Patent No. 2,460,641, August 1945.
4. Code of Federal Regulations. Food, Drug and Cosmetic Act. Pub. L. No. 75-717, 52 Stat. 1040 (1038), as amended 21 U.S.C. §§ 301 et seq. 1976.
5. International Standards Organization (ISO): Single-use containers for venous blood specimen collection. 6710, 1996.
6. National Committee for Clinical Laboratory Standards (NCCLS): Evacuated tubes and additives for blood specimen collection. H1-A4, December 1996;16(13).
7. United States Code Public Law. - Safe Medical Devices Act. Standards for New Medical Devices 101-629, 104 Stat. 4511 (1990).
8. Federal Register. US Food and Drug Administration Reference 21 CFR 820. Medical Devices; Current good manufacturing practice final rule; Quality system regulation. 1996;61(195).
9. International Standards Organization (ISO): Quality Systems - Medical devices - Particular requirements for the application of EN ISO 9001, EN ISO 13485, 2001.
10. Federal Register - Occupational Exposure to Bloodborne Pathogens; Needlestick and Other Sharps Injuries; Final Rule. 66:5317-5325.
11. Kasai M, Yamazaki S, Miyake S. "Blood collecting tube", U.S. Patent No. 4,985,026, January 1991.
12. Hatakeyama T. "Vacuum blood collecting production appts. - has tubular container storage feeder, pinhole checker, blood coagulant sprayer dryer, and separating agent injector". U.S. Patent No. 5,129,213, July 1992.
13. Antignani A, Cheng E, Evans J, Grippi, et al. "Method of using additive formulation and method for making tube with an additive formulation", U.S. Patent No. 6,187,553, February 2001.
14. Wayman DJ. Plastic blood tubes improve safety. *Plastics News*, 1994;June 6:52.
15. International Council for Standards in Hematology. Recommendations of the ICSH for EDTA anticoagulation of blood for blood cell counting and sizing. *Am J Pathol*. 1993;100:371-372.
16. National Committee for Clinical Laboratory Standards (NCCLS): Collection, Transport, and Processing of Blood Specimens for Coagulation Testing and General Performance of Coagulation Assays. H21-A3, December 1998.
17. Adcock, DM, Kressin DC, Marlara RA. Effect of 3.2% vs 3.8% sodium citrate concentration on routine coagulation testing. *Am J Clin Path*. 1997;107:105-110.
18. Narayanan S. Inhibition of in vitro platelet aggregation and release and fibrinolysis. *Ann Clin Lab Sci*. 1989;19:260-265.
19. Lin F-C, Cohen R, Losada R, et al. Cellular sedimentation and barrier formation under centrifugal force in blood collection tubes. *Lab Med*. 2001;32:588-594.
20. Pradhan S, Narayanan S, Lin FC. "Blood partitioning composition". U.S. Patent No. 4,994,393, February 1991.
21. FDA Web Site. Available at: <http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfPMN/pmn.cfm>. Accessed on February 18, 2003.