Electrophoresis

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Electrophoresis combines the prefix "electro," referring to electricity, and "phoresis," which comes from the Greek verb "phoros" and means "to carry across." Thus, electrophoresis means that electricity will carry something across. That "something" includes DNA, ribonucleic acid (RNA), genes, pharmaceuticals, metabolites, nucleotides, and peptides released by lysed cells. The electricity, acting on the surface charge of the substance of interest, carries it across a porous membrane at a speed proportional to the surface charge and the strength of the applied electrical field. Coupled with DNA copying techniques, this offers a powerful means of identifying a plethora of conditions and ailments. In situations where the molecules cannot be adequately detected, specific antibodies are mixed with the sample materiel. These antibodies attach themselves to the molecule—for example, a particular protein associated with HIV—and can be seen. Staining is sometimes used to increase visibility.

The application of electrophoresis has led to the development of a number of virtually error-free testing methods, such as Northern and Southern blotting, and the more commonly known Western blotting, used as a confirmatory test for a number of serious illnesses, including hepatitis and AIDS.

Current Technology

The basic principle of electrophoresis is simple, as is the equipment needed to comprise a system. The most basic electrophoresis system consists of a chamber or cell, a membrane, an incubator or oven, and a power supply. The chamber contains two buffer compartments, one at each end of the chamber. A bridge between the two compartments supports a membrane, typically cellulose acetate or some type of gel.

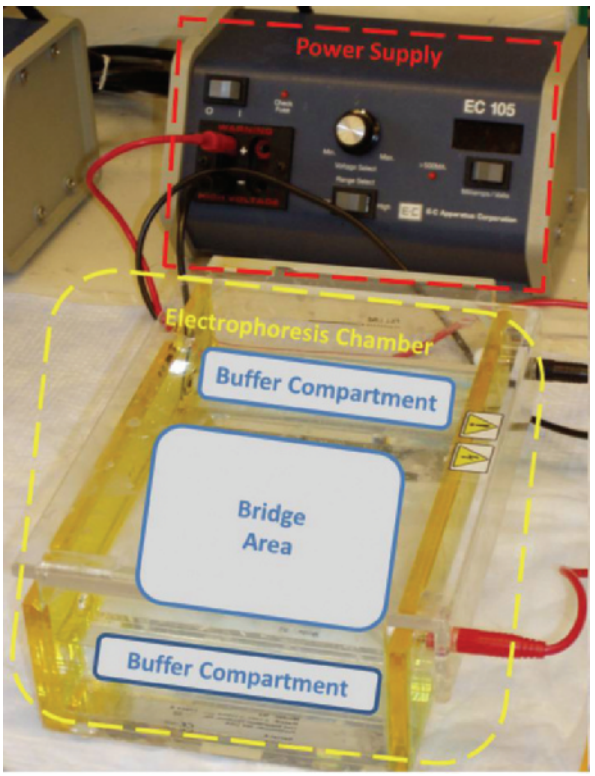

Figure 1. Gel electrophoresis apparatus. Source: http://commons.wikimedia.org/wiki/File:Gel_electrophoresis_apparatus.JPG

The membrane is prepared by using a pipette or microsyringe to inject a small amount of marker material, unknown sample material, and normal sample material in a series of "lanes" at one end of the gel. The preparation of the unknown sample material and the method of preparation of these samples vary at the discretion of the lab technician. The lab tech places the membrane over the bridge so that each end of the membrane contacts the buffer in its respective chamber and then connects the power supply with the negative terminal connected to the end containing the samples. The tech sets the power supply for a particular voltage (or current) and time, and allows the separation to occur.

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During electrophoresis, protein, DNA, or RNA segments in the samples at the bottom of each lane in the membrane migrate from negative to positive until equilibrium is reached and the segments remain in position. The process typically takes 20 minutes to an hour. There are dozens of variables that affect the outcome, including: the membrane (whether it is cellulose acetate, polyacrylamide, or some other gel material), the samples, the size and shape of the molecule of interest, the ionic strength of the buffer, the temperature, and of course, the voltage and duration of the electrical power application. Since these variables are unique to each test, specific electrophoresis tests use designated buffers, at particular temperatures, and various materials for the membrane. For example, in performing electrophoresis on cerebral spinal fluid (CSF) to diagnose multiple sclerosis, there are 15 distinct protein bands, a thin-layer agarose gel, and a buffer with a pH of 8.6. However, separations of large proteins and nucleic acids in hemoglobin most commonly use a standard agarose gel membrane and a buffer with a pH of 9.8.

After the set time, the tech disconnects the power supply and removes the membrane from the chamber. In most cases—but only when dictated by the test's protocol—the tech will dry or stain the membrane prior to examination. The tech then studies the lanes and compares the patterns in the unknown sample with the patterns in the marker materiel and normal sample materiel. Depending on the specifics of the test, there may be up to 20 "bands" or spots in each lane, and each band may be of a different size. This comparison of the bands' size and placement determines the test interpretation and the result. Alternatively, the tech may use a dedicated densitometer to determine the amount of light transmitted or reflected by each band.

How to Manage the Device

The biomed manager has choices in device management. Maintenance of electrophoresis systems that are visually interpreted by the lab tech can be in-house since only the incubator and power supply require attention. The remainder of the components—the chamber and membrane—require no maintenance and are simply replaced when broken. If the tech uses a densitometer, the options are different. Some densitometers are quite advanced, utilizing computer technology to remember and compare many different band patterns, which automates analysis. These densitometers may be best maintained through an annual service contract that includes periodic software and pattern upgrades. Most of the time, the original equipment manufacturer (OEM) contractor will want to cover the entire system for one price, while other contractors are willing to cover just the densitometer and leave maintenance of the incubator and power supply to the in-house biomed staff. Contract negotiations should cover these options, and ideally, contract proposals should offer coverage on an "a la carte" basis.

Regulations

There are no national regulations specific to electrophoresis. However, due to the minimal risk of electrical shock from either the power supply or the overall apparatus, the general duty clause of the Occupational Safety and Health Act (29 CFR) applies. This clause—section 5(a)(1) of the act— requires the employer to "furnish to each of his employees employment and a place of employment which are free from recognized hazards that are causing or are likely to cause death or serious physical harm to his employees". Laboratory and healthcare facility accreditation guidelines also apply.

Risk Management Issues

As noted, electrical shock to the operator is a possibility. The risk of inaccurate results, such as indeterminate or false negatives, also exists since so many variables depend on the lab tech. Likewise, the mitigation of these risks rests primarily with the tech.

Troubleshooting

It's up to lab tech to spot and correct the most common problems with electrophoresis. Of all the tests found in the laboratory today, this one probably requires the most technical skill on the part of technicians since they are responsible for the sample preparation, selection of buffers and membranes, voltage and current settings, and sample staining.

The three items of interest to the biomed are the incubator, power supply, and densitometer— all of which are all quite reliable. The incubator and power supply, in particular, should be no challenge to the trained biomed.

Incubator and power supply failures are quite rare. When they happen, they are usually hard failures, such as incubators that will either not heat or overheat, and power supplies that provide no output or indication of output on the display device, timer failure, and open leads from the power supply to the membrane. If not

detected during the electrophoresis setup, power supply problems manifest themselves by a lack of pattern migration during electrophoresis. A schematic is not required if the biomed is familiar with the general design of the incubators and power supplies.

Maintaining the densitometer is less straightforward. As mentioned earlier, maintenance of the device is often contracted since it contains proprietary software, and software upgrades can be included in the service contract. If maintenance is performed in-house, good service literature—including functional descriptions, block diagrams, schematic diagrams, and circuit-board layouts—is critical.

Future Development of Electrophoresis Systems

The forecast here is on three fronts. First, pulsed field electrophoresis (PFE) uses a switch connected to the output of the power supply to change polarity of the power applied to the membrane on a preprogrammed basis. The DNA molecules then uncoil and orient in one direction. When the polarity is reversed, the molecules reorient themselves to the new polarity. The large DNA molecules take longer to realign than the smaller ones, so there's a separation by size. This separation could not be accomplished with conventional electrophoresis. Applications based upon this variant are under development.

Second, a new electrophoresis technique—called "two-dimensional electrophoresis" or "2 DE"— is proving to be useful. This technique first requires the membrane to be exposed to isoelectric-focusing electrophoresis in one direction, then the electrical field is changed by 90 degrees, and gel electrophoresis occurs perpendicularly to the first. The resulting bands then have a two-dimensional character to them. This technique requires computerized scanning to interpret the information, and it may wind up in the laboratory of the future.

Finally, refinements in both optical technology and computer programming are expected to further improve membrane analysis. Integrated systems using higher-speed and more feature-packed computers will integrate densitometry and provide data analysis capabilities that we only now dream of. ■

It Started With Some Clay

In 1807, Ferdinand Friedrich Reuss, a German doctor, recorded the first-known observation of clay particles, dispersed in water, moving under the influence of a spatially uniform electric field provided by a voltaic pile (a crude battery). Through these experiments, Reuss determined the existence of "barriers" in forms of sand and clay between the poles of the battery. Although Reuss was a doctor of both medicine and surgery he was more of a general scientist, and he conducted more sporadic experiments and published several papers on this and related observations until 1821. But Reuss was relatively obscure in the western European scientific community and a disastrous fire destroyed most of his research papers, so his work got little notice. (There is also some historic speculation that, although this was an interesting phenomenon, Reuss lacked a practical application for it, so he turned his attention elsewhere.)

Reuss' observations lay virtually dormant, except for a few experiments by other scientists of the era, such as Johann Wilhelm Hittorf and Walther Nernst, who measured the behavior of small ions moving through liquid solutions when influenced by an electric field.

In 1930, Arne Tiselius rediscovered this occurrence and wrote about it in his doctorate thesis, "The Moving-Boundary Method of Studying the Electrophoresis of Proteins." Tiselius continued his experiments on the application of physics and physical methods to general biochemical problems, winning the 1948 Nobel Prize in Chemistry. A major result of his experiments was the development in 1937 of a "Tiselius apparatus," a U-shaped assemblage of glass tubes constituting the first true electrophoresis device. This device not only enabled new applications of this developing technology in analyzing chemical mixtures, it prompted some major chemical research centers to acquire their own Tiselius apparatuses and conduct their own experiments.

Tiselius and his apparatus made a profound impact on both biology and biochemistry. But he was not alone. From the 1940s to the 1960s, other scientists developed techniques using filter paper and various gels as supporting media for electrophoresis. These more sophisticated gels allowed the separation of biological matter based on small chemical and physical differences, giving rise to the field of molecular biology and forming the basis of sophisticated electrophoresis tests, including protein fingerprinting, Southern, Western, and similar blotting procedures, and DNA sequencing.