Oct. 22, 1968 W. E. OLIVA ET AL 3,407,133

EXPENDABLE ELECTROPHORESIS APPARATUS

Filed June 18, 1965 2 Sheets-Sheet 1

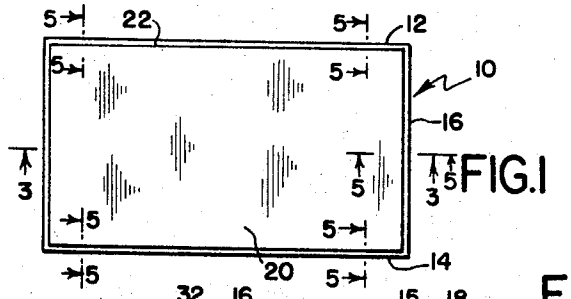


FIG. 1

FIG. 5

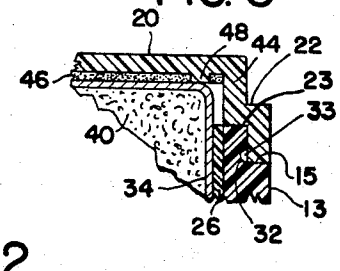
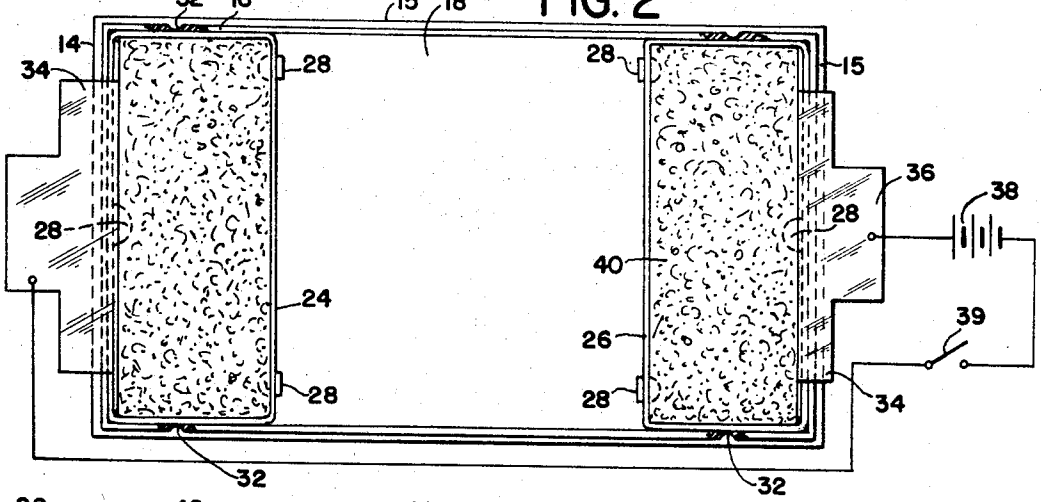


FIG. 2



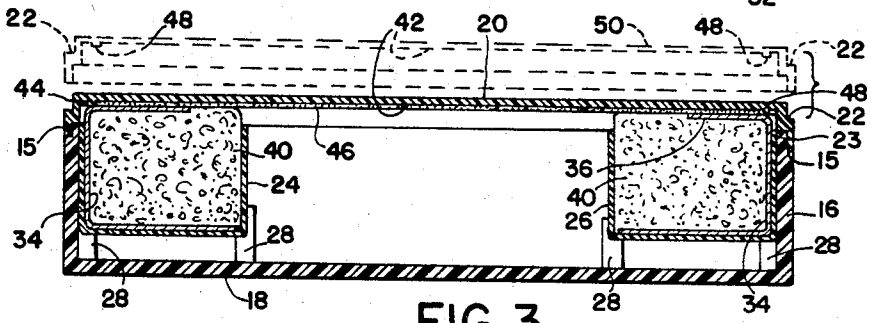


FIG. 3

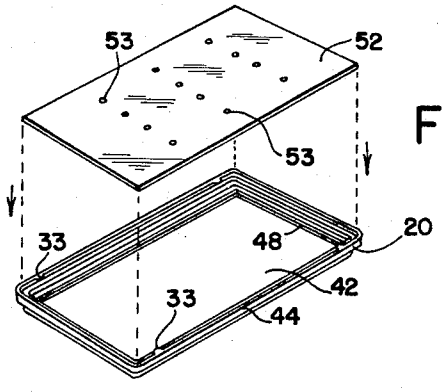


FIG. 4

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EXPENDABLE ELECTROPHORESIS APPARATUS

Filed June 18, 1965 2 Sheets-Sheet 2

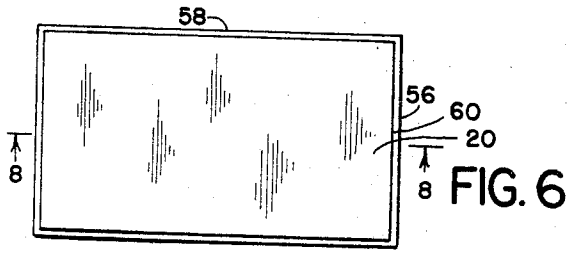

FIG. 6

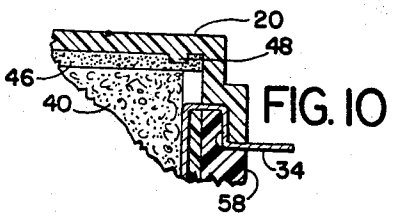

FIG. 10

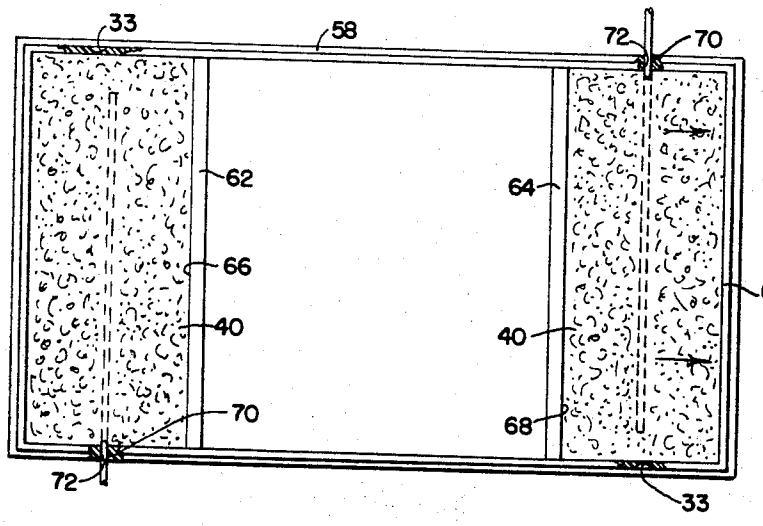

FIG. 7

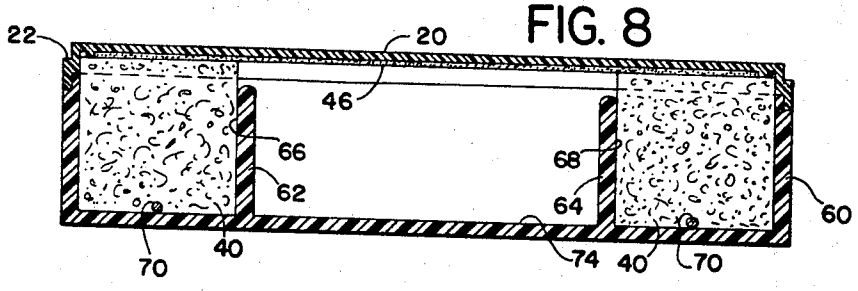

FIG. 8

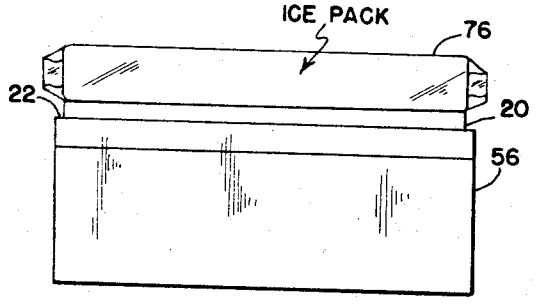

FIG. 9

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3,407,133

EXPENDABLE ELECTROPHORESIS APPARATUS

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Filed June 18, 1965, Ser. No. 465,059
12 Claims. (Cl. 204—299)

This application relates to the separation of mixtures by differential migration of components through a transport medium in an electric field and more particularly to a new and improved expendable plastic electrophoresis test unit for particle separation in a buffer system to which direct current is applied.

Various types and sizes of electrophoretic test apparatus have been provided in the past for separating components of a mixture wherein each material has its own particular charge which differs from the others in extent and sign. The separation and consequent grouping of materials electrophoretically normally occurs within a buffered support medium wherein those particles of a test sample that have a greater charge will migrate at a faster rate and further in a given time than those of lesser charge. After particle grouping, visual identification of the materials in the test sample is determined by known means such as for instance colorimetric analysis. In the past the procedures described required the use of expensive and relatively complicated apparatus which demanded the presence of technicians possessed of a high degree of skill and available time for detailed attention to the preparation, operation and subsequent clean-up of the test apparatus. All of these steps, together with the cost of the equipment required, have hindered the potential application of electrophoresis as a suitable test procedure, especially in the clinical field where the use of expendable apparatus has been so well accepted for a variety of reasons, economic and otherwise. In the area of activity referred to above, it has become increasingly useful to be able to determine for example the identification of the individual isozymes of lactate dehydrogenase. It will be understood however that this is but one application to which electrophoretic separating is adaptable and is mentioned here for illustrative purposes only. The use of inexpensive, expendable, self-contained electrophoresis equipment will enable the relatively small clinic to use diagnostic procedures which heretofore have been fairly well restricted to use by large commercial and institutional laboratories.

In addition, electrophoresis apparatus presently used is of such design that each setup involves the gathering together and assembly of various pieces of costly and sometimes cumbersome equipment. To accommodate more than one test at a time requires the possession and use of duplicate apparatus, compounding the expense and increasing the probability of error due to improper disassembly and cleaning after use. The present invention makes available for clinical use a kit-type expendable single use apparatus. This quality enables the operator to normally work with a much smaller piece of equipment and further releases him from the problems of assembly and cleanup mentioned above necessitated by reuse of any piece of laboratory equipment. With all necessary component materials and equipment supplied in a single package including a plurality of lids adapted to contain transport medium many of the above problems are obviated.

It is therefore a primary object of the present invention to provide a new and improved, relatively inexpensive, expendable electrophoresis test apparatus.

A further object of this invention is to provide a means of performing electrophoretic tests whereby all necessary test components for the particle separation procedure are provided in a single kit.

Still another object of this invention is to provide a new and improved means for supporting a pre-poured gel medium for passage of electric current therethrough for particle migration therein.

Another object is to provide a new and novel means for depositing test samples upon a suitable gel support medium for electrophoresis particle migration.

Another object is to provide an improved means of providing current through a buffer in a gel medium in an electrophoresis test apparatus.

A feature of the present invention is the provision of a compact, disposable electrophoresis test apparatus formed of a plastic material and including a base chamber with removable buffer vessels positioned therein and having electrode means and a lid adapted to position a gel support medium between the buffer vessels.

Another feature is the provision of an integrally formed multi-reservoir electrophoresis chamber wherein a pair of spaced buffer vessels are provided with a buffer in contact with a gel medium provided in a plastic lid for imposition of direct current upon the system with consequent passage of the current through the gel medium.

Still another feature is the provision of an integrally formed multi-reservoir electrophoresis chamber wherein a pair of spaced electrode containing buffer vessels are provided with a wick to maintain a buffer in contact with a gel medium poured and set in a plastic lid for imposition of direct current upon the system with consequent passage of the current through the gel medium.

A further feature is the provision of a plastic lid for an electrophoresis chamber, the lid being adapted to contain a gel support medium and formed to receive a further lid thereon in stacked relationship for compact shipping of a plurality of lids with a single base chamber.

Still another feature of the invention is the provision of a template for forming predetermined spaced test sample wells in a solid gel medium.

Another feature of the invention is the provision of foil electrodes formed to substantially fit a pair of buffer vessels and having means for connection to a source of current.

The above and other objects and features of the present invention will become apparent from the following description and accompanying drawings forming part of this application.

In the drawings:

FIGURE 1 is a plan view of the electrophoresis kit of the present invention;

FIGURE 2 is an enlarged plan view of the apparatus of FIGURE 1 with the lid removed;

FIGURE 3 is a horizontal sectional view taken along lines 3—3 of FIGURE 1;

FIGURE 4 is a perspective illustration of the lid and template of the present invention;

FIGURE 5 is a typical partial sectional view as taken generally along, and in the direction of any of the lines 5—5 of FIGURE 1;

FIGURE 6 is a plan view of a further embodiment of the present invention;

FIGURE 7 is a plan view of the device of FIGURE 6 with the lid removed;

FIGURE 8 is a horizontal sectional view taken generally along, and in the direction of lines 8—8 of FIGURE 6;

FIGURE 9 is a side view of the apparatus of FIGURE 6; and

FIGURE 10 is a partial sectional view identical to FIGURE 5 showing the foil electrode in the operable position.

Referring now particularly to the drawings wherein like reference characters indicate like parts, the electrophoresis kit of the present invention shown in FIGURE 1 is indicated generally by the reference numeral **10** and includes a substantially rectangular electrophoresis chamber **12** having sidewalls **14** and end walls **16** surrounding, and extending upwardly from, a flat base portion **18** (see FIGURE 3). Each of the walls **14** and **16** is provided with an area of reduced thickness at the upper edge portion defining an L-shaped lid rest **15** in the walls **14** and **16**. The chamber **12** is shown with a lid **20** resting upon the top portion thereof. The lid **20**, as illustrated in more detail in FIGURE 3, is formed with an upper portion of reduced outer peripheral dimension defining an outer ledge **22** to accommodate stacking additional lids one upon the other for shipment as a compact unit. An inner ledge **23** is likewise formed on the lid **20** to rest upon the chamber walls upon assembly of the kit. The top portion of the walls **14** and **16** together with the inner ledge **23** thus provide complementary engageable surfaces as shown in FIGURES 3 and 5.

FIGURE 2, an enlarged view of the unit **10** of FIGURE 1, shows the chamber **12** with lid **20** removed. Positioned within the chamber **12** are a pair of substantially rectangular buffer vessels **24** and **26**. The vessels **24** and **26** are open at the top and positioned parallel to the end walls **16** of the chamber **12**. Vessel support pads **28** are provided upon the base **18** and extend upwardly therefrom to provide support for the vessels **24** and **26** within chamber **12** maintaining the vessels **24** and **26** in predetermined vertical position within the chamber **12**. The support pads **28** for the vessels **24** and **26** further hold them in spaced longitudinal relationship within the chamber **12** until removed by lifting from the chamber **12**. Recesses **32** are formed in the outer surface of the side walls **14** to receive the nubs **33** formed on the lid **20** to hold the lid **20** in its closed position upon the chamber **12** during electrophoresis. The vessels **24** and **26** may be provided with integrally formed outwardly projecting hanger tabs (not shown) to allow suspension of the buffer vessels within the chamber **12** from the walls thereof. A foil electrode **34** of suitable electrical conducting properties is provided for retention within each of the buffer vessels **24** and **26** and is shaped to substantially fit within the vessels **24** and **26** as seen in FIGURES 2 and 3. Each foil electrode **34** has an outwardly extending tab portion **36** which is adapted to protrude from the chamber **12**. The tabs **36** are connected to a suitable source of direct current **38** through switch **39**. The tabs **36** are illustrated in their extended outfolded operable position in FIGURE 2. It will be understood that although foil electrodes are illustrated, other known electrodes such as nichrome or platinum wire (as seen in FIGURE 8) may be used. Normally a pair of electrodes are provided with each lid **20** supplied with the kit and may be contained within the vacant section of the chamber **12** between the buffer vessels. The electrode may in fact be painted or printed in the buffer vessels **24** and **26** without departing from the spirit and scope of the present invention.

When assembled as a kit for distribution to the user the foil electrode tabs **36** may be folded as seen in FIGURE 3 over the top of a sponge wick **40** provided in each of the buffer vessels **24** and **26** of the unit **10**. The sponge wicks **40** of suitable porosity carry and convey a buffer solution poured into vessels **24** and **26** prior to electrophoresis to provide the buffer communication between the vessels **24** and **26** and the gel support medium, which will be discussed in the description of FIGURE 3. The sponge wicks **40** are shown formed to fit the vessels **24** and **26** conforming substantially to the internal dimensions of the vessels and upon saturation with buffer solution contact the gel within lid **20** upon closure of the chamber **12**.

In FIGURE 3 a lid **20** is illustrated in position upon the chamber **12** to effectively enclose the chamber **12** to aid in prevention of evaporation therefrom. The lid **20** is further formed to receive and contain a suitable gel medium **46** by providing a gel reservoir **42** when positioned for the receipt of gel therein. The gel reservoir **42** within the lid **20** is defined by the vertically extending peripheral wall **44** of lid **20**. It will be understood that the gel medium may be pre-poured and thus packaged and provided by the manufacturer with the lid **20**; or alternatively it may be poured by the user. With the lid **20** in position upon the chamber **12**, the gel medium at the end portions of lid **20** rests in electrical contact upon the sponge wicks **40**. The lid, buffer vessels and sponges are dimensionally proportioned so that the gel may adequately contact but not unduly compress the sponges. Vertically extending gel retainers **48** may be formed upon the inside surface of the lid **20** extending laterally thereacross in spaced relationship thereon to aid in holding the semi-solid support medium **46** in position within the lid **20** when inverted and locked in position upon chamber **12** for operation of the apparatus. A second lid **50** is illustrated in phantom in FIGURE 3 to show the stacked relationship between the lids. The lids **20** and **50** are of like construction and are thus adapted to form a compact package for shipment and storage. In this manner several lids may be provided with a single chamber **12**.

FIGURE 4 shows a lid **20** in position to receive a gel pour therein. Nubs **33** are integrally formed on the inner walls of the lid **20** and are provided to engage the depressions **32** in the outer surface of chamber **12** when the lid is placed in position thereon. In this manner a relatively tight fit is accomplished to hold the gel medium **46** on sponges **40** and further to inhibit evaporation during electrophoresis.

A template **52** as seen in FIGURE 4 is used to locate test sample well formation in the gel support medium within lid **20**. Template **52** is illustrated with a plurality of openings **53** through which a suitable well drilling tool may be passed to form the wells.

FIGURE 5 shows, in an enlarged fragmentary manner, a portion of the lid **20** in place upon the chamber **12**. The foil electrode **34** is shown folded over the sponge wick **40** with foil tab **36** in its non-operable position. The foil electrode **34** as provided with the unit **10** lines the inner side wall and bottom of each of the buffer vessels **24** and **26** and is adapted to be folded outwardly and pass between the top surface of the wall **44** of lid **20** and top of the wall of chamber **12** (as shown in FIGURE 10). Upon folding the foil **34** to operable position the sponge wick **40** contacts the buffered gel medium **46**.

FIGURE 6 is a plan view of a modification of the electrophoresis test unit of FIGURE 1 and shows a lid **20** provided upon a chamber **56** having side walls **58** and end walls **60**. The chamber **56** has integrally formed divider walls **62** and **64**, as seen in FIGURES 7 and 8, which define with walls **58** and **60** a pair of buffer vessels **66** and **68** in longitudinal spaced relationship therein. The walls **62** and **64** are vertically coextensive with the

outer walls **58** and **60** of the chamber **56**. Sponge wicks **40** are positioned within the buffer vessels **66** and **68** to convey buffer to a gel medium **46** which is contained in the lid **20**. A wire electrode **70** is provided through each opening **72** in the side walls **58**. The lid **20**, which is adapted for complementary interlocking engagement with the upper peripheral edge of the side walls **58** of chamber **56**, is of similar construction to that described in reference to unit **10** of FIGURE 3.

FIGURE 8 shows the lid **20** in position upon the chamber **56** and is a further view of integrally formed dividers **62** and **64** which separate chamber **56** into three compartments including the buffer vessels **66** and **68** and a vacant chamber **74** therebetween.

FIGURE 9 is a side view of the electrophoresis kit of the present invention showing an ice pack **76** positioned upon the top of the lid **20** to serve as a coolant during electrophoresis.

FIGURE 10 is a view similar to FIGURE 5 and shows the foil electrode **34** in its folded out operable position whereby it resides between the complementary engaging wall surfaces of the lid **20** and chamber **56**.

In order to electrophoretically separate materials by differential migration in an electrically conductive buffer system subjected to flow of current therethrough utilizing the disposable test apparatus of the present invention, the test sample, which may contain proteins, peptides, and amino acids, or like materials subject to electrophoretic particle separation, is deposited within a buffered support medium. The support medium described and illustrated herein is gel, although other known medias such as filter paper or cellulose acetate may be utilized. The gel, which may have been pre-poured by the supplier or alternatively prepared and poured by the user is contained within a lid **20** provided with the test kit of the present invention. The test sample is placed at predetermined positions in contact with the solid gel. If it is desired to place the sample in a predetermined location in the gel, a template **52** may be used to locate a well position wherein a suitable removal device is used to take gel from the lid **20** through openings **53** formed in the template **52**. The buffered gel, as discussed above in the description of FIGURE 5 is maintained within the lid **20** by wall **44** and further by gel retainers **48**. In this manner, when the test sample perfuses the solid gel, the lid is ready to be inverted and positioned upon the chamber **12**. Since the buffer vessels **24** and **26** are provided with wicks **40** which rest with their top portions substantially in vertical alignment with the upper edges of the chamber **12**, the gel medium will rest in electrical contact with the buffer saturated sponge wicks **40** with the lid **20** in place for operation. Prior to lid placement, a buffer solution is poured over the wicks **40** in vessels **24** and **26**. Since the gel medium in lid **20** is prebuffered, with the lid in place there exists a system or path of continuous buffer from one vessel **24** through the gel medium **46** to the second vessel **26**, effectively completing a circuit through switch **39**. To initiate electrophoresis direct current is applied through the components of the system. This is accomplished by connecting the foil electrodes **34** which are provided within the buffer vessels **24** and **26** to a suitable current source **38**. It will be understood that passage of current causes flow of buffer ion salts to migrate through the buffer-gel system. In operation heat is generated within the system as an incident to the imposition of direct current on the system. Such heat, if not adequate dissipated, would interfere with the proper function of the system. In FIGURE 9 ice pack **76** is positioned upon the unit **10** to dissipate heat generated within the chamber. It will be understood that any suitable coolant means may be employed to accomplish the above stated heat exchange objective. The coolant could be positioned in the vacant center chamber between the vessels.

The potential is applied through the system for a predetermined length of time, after which a suitable method of analysis is employed to locate and identify the electrophoretically separated constituents of the test sample. It will be understood that the direction and rate of particle migration are functions of the magnitude of the field, the charge on the particles, the pH of the buffer, and other factors such as particle size, viscosity and the like. The term particles is used to denote material of extremely small dimension, e.g. molecules such as amino acids.

Thus is provided a relatively inexpensive, expendable electrophoresis apparatus which includes all of the necessary parameters for testing and is so constructed as to offer a compact unit which may include several gel lids with a single disposable electrophoresis chamber.

Having thus described the invention, what is claimed is:

1. An expendable electrophoresis test kit comprising:
- a plastic chamber having means defining a pair of spaced buffer solution vessels therein,
- means to position a buffered gel support medium between said buffer solution vessels and in electrical contact with the buffer solution in each vessel,
- said last named means including a generally dish shaped removable plastic lid portion positioned upon said chamber,
- means on the under surface of said lid for retaining said gel support medium,
- means including electrodes within the buffer solution vessels to provide passage of current through said buffer solution buffered gel medium to permit charged particle migration within said gel medium for particle group identification therein.

2. An expendable electrophoresis test kit comprising:
- a plastic chamber having a pair of spaced inner walls defining a pair of spaced buffer solution vessels therein,
- means to support a buffered gel medium bridge between said buffer solution vessels in electrical contact with the buffer solution in each vessel,
- said last named means including a dish shaped plastic lid portion,
- means including electrodes within the buffer solution vessels for passage of current through said buffer solution and buffered gel medium to provide charged particle migration within said gel medium, and a ledge formed upon said lid to provide for stacking an identical lid in nested relationship thereon to provide compact plurality of lids with a single base chamber.

3. An expendable electrophoretic test kit for particle migration within a buffered gel medium comprising:
- a substantially rectangular open top plastic chamber,
- a pair of longitudinally spaced plastic vessels removably positioned within the tank, each of said vessels provided to receive a buffer solution therein,
- an electrode within each of said vessels to afford electrical potential to the buffer solution upon connection to a source of direct current,
- a removable lid positioned in said chamber and adapted to hold a buffered gel support medium on its under surface, over and between said vessels in electrolytic contact with the buffer solution therein whereby passage of current within the system promotes electrophoretic particle migration of a test sample within the buffered gel support medium.

4. An expendable electrophoresis kit for particle migration within a buffered gel medium comprising:
current flow therethrough comprising:
- an open top plastic base chamber,
- means within the chamber defining a pair of spaced plastic buffer vessels therein,
- an electrode within each of the buffer vessels and adapted for connection to a source of direct current,
- buffer carrying wick means provided within said vessels,
- and a plastic lid removably positioned upon the chamber and including gel medium support means on

its under surface for providing a buffered gel bridge between the wicks whereby imposition of potential between the vessels through the gel medium induces particle migration therein.

5. An expendable electrophoretic test kit for particle migration within a buffered gel medium comprising:
a substantially rectangular open top plastic chamber,
a pair of longitudinally spaced plastic vessels removably positioned within said chamber and open at the upper portion thereof, said vessels provided to contain a buffered solution therein,
a foil electrode positioned within each of said vessels to provide electrical potential to the buffer upon conupon connection to a source of direct current,
a buffered gel support medium,
a lid removably positioned to enclose said chamber and to receive and maintain said gel support medium on its under surface over said buffer vessels in current passing relationship whereby passage of current through the system promotes electrophoretic particle migration of a test sample within the gel.

6. An expendable electrophoretic test kit for particle migration within a buffered gel medium comprising:
a substantially rectangular open top plastic chamber,
a pair of longitudinally spaced buffer vesels removably positioned within said chamber and open at the top and provded to contain a buffered solution therein,
a foil electrode positioned within each of said vessels to provide electrical potential to the buffer upon connection to a source of current,
a porous wick within each of said vessels,
a buffered gel support medium,
a lid removably positioned to enclose said chamber and to receive and maintain said gel support medium on its under surface over said buffer vessels in passing relationship whereby passage of current through the system promotes electrophoretic particle migration of a test sample within the gel.

7. An expendable electrophoresis kit comprising:
a substantially rectangular open top plastic chamber,
a pair of vertically extending lateral walls integrally formed adjacent each end of said chamber and longitudinally spaced to define a pair of buffer vessels therein, said walls being vertically co-extensive with the outer walls of said chamber whereby said vessels are adapted to receive a buffer solution therein,
a wick within each of said buffer vessels to hold the buffer solution,
a foil electrode provided within each of said vessels having means for connection to a source of potential to pass electrical current through the buffered solution in said vessels,
a lid portion removably positioned upon said chamber providing a cover therefor to prevent evaporation therefrom and means formed within said lid portion to hold a buffered gel medium over said vessels in electrical contact with said buffer solution whereby flow of current through the system promotes particle migration of a test sample within the gel support medium.

8. An expendable electrophoresis kit comprising:
a substantially rectangular open top plastic chamber,
a pair of vertically extending lateral walls integrally formed adjacent each end of said chamber and longitudinally spaced therein to define a pair of buffer vessels, said walls being vertically co-extensive with the outer walls of said chamber, said vessels further adapted to receive a buffer solution therein,
a wick within each of said buffer vessels to hold the buffer solution,
a foil electrode provided within each of said buffer vessels having means for connection to a source of potential to pass electrical current through the buffered solution in said buffer vessels,
a plastic lid portion removably positioned upon said chamber,
a buffered solid gel support medium provided within said lid,
means formed within said lid portion to hold the buffered gel medium on its under surface over said vessels in electrical contact therewith whereby flow of current within the system promotes particle migration of a test sample within the buffered gel support medium.

9. An expendable electrophoresis kit comprising:
a substantially rectangular open top plastic chamber,
a pair of vertically extending lateral walls integrally formed adjacent each end of said chamber and longitudinally spaced to define a pair of buffer vessels therein, said walls being vertically co-extensive with the outer walls of said chamber whereby said vessels are adapted to receive a buffer solution therein,
a wick within each of said buffer vessels to hold the buffer solution,
a foil electrode provided within each of said vessels having means for connection to a source of potential to pass electrical current through the buffered solution in said vessels,
a plastic lid portion removably positioned upon said chamber,
a buffered gel support medium provided within said lid,
means formed within said lid portion to hold the buffered gel medium on its under surface over said vessels in electrical contact therewith whereby flow of current within the system promotes particle migration of a test sample within a buffered gel support medium and a template for predetermined test sample well location within the gel support medium.

10. An expendable gel support retaining lid for an electrophoresis test chamber having a pair of spaced buffer vessels with electrodes therein comprising:
a substantially flat base portion,
a vertically extending wall formed at the periphery of the base portion,
gel support medium retaining means provided within the base to hold the gel within a lid upon inversion thereof for positioning the gel in electrical contact with the buffer vessels, and locking means on the lid adapted to engage the chamber to effectively enclose the chamber against evaporation therefrom during electrophoresis.

11. A gel medium support retaining lid for an electrophoresis test chamber having a pair of spaced buffer vessels with electrodes therein to position the buffered gel support medium over and between the spaced buffer vessel comprising:
a substantially flat base portion,
a peripheral wall extending vertically from said base portion to provide a generally dish shaped member,
gel retaining means integrally formed within said member,
fastening means upon said wall to lock the lid upon the test chamber during electrophoresis and further seal the chamber minimizing evaporation therefrom.

12. In combination with an expendable electrophoresis chamber including a chamber having a pair of spaced buffer vessels and including electrode means within the vessels adapted for connection to a source of direct current, a gel medium support retaining lid to hold a buffered gel between the vessels in electrical contact therewith comprising:
a base portion having an upwardly extending peripheral wall, and of a configuration to cover the chamber,
locking means formed upon said peripheral wall to hold the lid in position upon the chamber during electrophoresis to inhibit evaporation therefrom.

means provided within the base portion to maintain solid gel in position thereon when said base portion is in its inverted operable position upon the unit, said base portion having means formed upon the wall defining a peripheral for receiving in stacked relationship an additional lid thereon.

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