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17. CLINICAL ELECTROPHORESIS ON CELLOCLEAR AGAROSE PLUS
Cellogel Electrophoresis Co. originates from the industrial research activities of Dr G.B. Del Campo who established the company Chemetron, specialised in chromatography and electrophoresis, immediately after graduating in 1958. In the same year Dr Del Campo set up the company Chemetron Chimica which specialised in the production of kits for clinical chemistry analysis of blood, urine, etc. The two companies operated for 13 years (1958-1971), without Italian or European competitors, in the field of clinical chemistry.

Chemetron’s most important success of was the invention of Cellogel, patented internationally by Dr Del Campo in 1963 and distributed in 36 countries worldwide. Cellogel was, in fact, the product favoured both by researchers and by users who utilised it for routine analyses. The success of Cellogel was totally due to the numerous applications proposed by Dr Del Campo’s research staff. The magazine Chimica Clinica Acta published works on Cellogel by authors who worked in the most famous European, North American, Japanese, Australian and Argentinian research institutes. Even the sacred texts of Internal Medicine such as Introzzi in Italy, Gras in Spain and Margni in Argentina quoted Cellogel in the chapters on electrophoresis and immunofixation. The official pharmacopoeia, both Italian (FU) and French, English and American (USP) mention the analytical methods of Cellogel for the control of proteins such as human albumin, pure immunoglobulins, etc. The best known international journals, Clinical Chemistry, Biochimica Clinica, etc. published works by researchers who used Cellogel for their immunological techniques, for the separation of isoenzymes or the determination of single proteins.

Cellogel was distributed by such important companies as: Whatman Ltd (U.K.), Serva Heidelberg (Germany), Fisher Scientific (USA), etc.

Important customers who have utilised Cellogel and published the results of their research have been, among others, the Carolinska Institute (Sweden), the Weizmann Institute (Israel), the chain of Max Planck Institut (Germany), MIT – Massachusetts Institute of Technology, Boston (USA).

In the mid-sixties new companies were established and started with the distribution of Cellogel, including Sebia (France), Atom and Biosystems (Spain), Labometer (Portugal), International Cientifica (Mexico) and other firms in South America (Venezuela, Brazil and Argentina).

In 1988 Dr Del Campo transferred the brands and patents to other companies among which Malta Chemetron which has produced Cellogel in recent years and then to the Cellogel Electrophoresis Company of Milan.

The new company has a programme covering the whole field of clinical electrophoresis which includes research and automation, aiming, above all, at quality and innovation with new products, in particular equipment for manual electrophoresis for small and large numbers of tests/hour, automatic equipment for high resolution electrophoresis and densitometer-scanner for rapid quantitative readings of electrophoretic migrations.
Since 1966 Prof. J. Kohn, the most important English author on clinical electrophoresis, at the VI International Congress of Clinical Chemistry in Basel had defined micro electrophoresis of serum proteins or similar electrophoresis with short migrations under 3 cm an “abuse of electrophoresis” and had denounced their diagnostic and also their analytical insufficiencies. Prof. Laurell did the same at the International Congress of Clinical Chemistry in Copenhagen in 1971; above all this author criticised the analytical value of 5-band electrophoresis, which group the numerous unseparated proteins in each zone and he disclosed some information about his high resolution agarose electrophoresis research programme (HRE) on serum proteins. To obtain a true analysis of the serum proteins Laurell recommended the determination of the quantity of single proteins using the technique of electro-immuno diffusion or Rockets. Laurell’s technique proposed optimising the analysis of the serum proteins and did not consider the fact that the main purpose of clinical electrophoresis was to discover monoclonal gammapathies in asymptomatic patients and not a perfect qualitative-quantitative chemical analysis of the proteins.

In 1979 Professors Laurell and Jeppson published their “proposed selected method” of high resolution electrophoresis of serum proteins on agarose in Clinical Chemistry, Vol. 25, No. 4, pages 629-638. It must, however, be said that the high resolution method for serum proteins had already been published in 1968 by Dr Del Campo in Chimica Clinica Acta. This was about the first method of high resolution electrophoresis on Cellogel RS (Cellogel Renal Studies) which separated between 13 and 23 protein fractions on the tested sera and also made possible the multi-fractionation of unconcentrated urine proteins and of unconcentrated liquor with deposits of 50-100 μl of a sample on the start point in the form of a drop (concentration of the proteins at the deposit point). Before Jeppson and Laurell’s publication the “Microlong” method on 5.7x14 cm and 5.7x17 cm Cellogel strips, with 8 micro deposits, migration length approx. 6 cm and Coomassie high-sensitivity stain was proposed in Chemetron News (1976).

Microlong on Cellogel was widespread in northern countries and, in fact, Drs T. Weber and K. Ojala of the Aurora Hospital, Helsinki, replied to Jeppson and Laurell’s publication in Clinical Chemistry, Vol. 26, pages 1754-5, demonstrating that it was possible to obtain high resolution utilising Cellogel with excellent diagnostic validity in a very simple and rapid way compared to the complicated method on agarose adoptable only in research laboratories but not in hospital laboratories for routine work and that Agarose was better suited to analysis, not to diagnosis. In the meantime in Italy the SIBioC commission on proteins published its recommendations according to which good quality electrophoresis of the serum proteins suitable for discovering the monoclonal bands, even below the limit of 1g/L, should be undertaken with semi-micro “elongated” electrophoresis on Cellogel with migrations up to 5.5 cm and using the usual stains such as Amidoblack.

It must be considered that there is a difference in the capacity of discovering small monoclonal bands between Microlong on Cellogel which used a micro deposit of 0.3 μl and reveals 90 ng/band with a Coomassie stain and the elongated semi-micro electrophoresis which deposits about 1 μl and reveals 250 ng/band with Amidoblack stain.
Towards the end of the eighties, everybody expected the European and American electrophoresis multinational companies would propose high resolution systems suitable for the routine of large laboratories with the aim of obtaining the maximum capacity of revelation of the monoclonal bands, and hence the most certain diagnoses of gammapathies without the risk of diagnostic omissions typical of micro electrophoresis with 20 mm migration. It must be noted that the discovery of monoclonal gammapathies in asymptomatic patients is completely chance and only occurs when a valid electrophoresis test of the serum proteins is prescribed.

Instead, what happened was that the Japanese multinational companies (Olympus, Jookoo, etc) who were extraneous to the tradition and western electrophoretic culture, proposed automatic systems which generally worked on a micro scale obtaining an unforeseeable success in hospital laboratories, to the detriment of the diagnosis of monoclonal gammapathies and harm to the patients.

The only exceptions were Malta Chemetron, who continued to insist on high resolution electrophoresis and the American company Panagel supported by the text “High Resolution Electrophoresis and Immunofixation” of 1994 by Dr F. Keren.

The situation of good quality electrophoresis has not improved in the nineties. An American multinational company introduced CZE (Capillary Zone Electrophoresis) to the market followed immediately after by a French company. The latter proposed a step system of miniaturized micro electrophoresis on agarose improperly named “automat d’electrophorese”.

Capillary Electrophoresis was able to perform 60-80 “fast electrophoresis” of serum proteins and “immuno-subtraction” of monoclonal components in substitution of immunofixation (IFE) for the classification and typing of monoclonal components.

To date immuno-subtraction performed on automatic capillary equipment leaves the users totally unsatisfied because it is a de-sensibilizing technique compared to IFE on Cellogel (Aguzzi and Rezzani high resolution method) or on agarose. But electrophoresis of the serum proteins by the Capillary method is also unsatisfactory, at least to the same extent as the micro electrophoresis on French agarose or on cellulose acetate applied on Japanese automatic systems. In fact with the aim of eliminating the numerous interferences of the non protein components of the serum the CZE worked with volumes of samples in the order of a nanoliter (1 nanoliter = 0.001 µl), so as to remain insensitive to interferences and to see, by means of the UV detector, only the protein fractions present in concentrations superior to 1 g/L without, however, guaranteeing the subtraction of the non-protein substances transported by, for example, albumin: it is ascertained that patients who have drunk a coffee or taken an aspirin or an antibiotic with peaks of absorption between 200 and 240 nanometers with the CZE give a much higher value of albumin than that given by an analysis carried out with the same CZE an hour before the medicines were taken. In electrophoresis on Cellogel no specific stain can colour the caffeine, aspirin or antibiotic transported, and, therefore the albumin value remains unaltered.

At this moment, the conclusion to be drawn is that the marketing of the multinationals continues to impose, despite the expectations of experts in electrophoresis and gammapathies, rapid systems meeting the generic desires for automation expressed by those laboratory directors more interested in the organisation of the analysis service and hence in precise delivery times of the results than in the quality and the diagnostic value of these analyses; and this regardless of expense, which is actually doubled or trebled in the passage from valid manual semi-micro elongated electrophoresis to the less valid automatic CZE electrophoresis or the semi-automatic micro on agarose. It is worth quoting an important publication by Xavier Bossuyt and Godelieve
These authors, after several comparisons of various models of CZE equipment and agarose, denounced the incapacity of the new CZE systems to reveal the monoclonal components, so much so that they decided to perform immunofixation directly on all patients who were referred to their laboratory for the first time with a prescription for an electrophoretic test having purpose of monoclonal gammopathy research.

The expectations of authors and particularly of experts in monoclonal gammapathies, have been frustrated because, until now, no multinational company has presented a valid automatic instrument for high resolution electrophoresis of serum proteins or for rapid immunofixation of a large number of samples, or for automatic electrophoresis of unconcentrated urinary proteins.

To meet this diagnostic gap our company has prepared rapid methods which meet the needs of organisations and deliver a high number of electrophoretic tests and IFE. We also offer a service for the training of valid laboratory technicians as well as a complete service of technical assistance on-line. All this is aimed at the diagnosis of monoclonal gammapathies with cognizance of the facts.

We should not forget, among other things, the results of the International Multicenter Trial to Investigate Monoclonal Components of 1991. 4788 sera were tested. Italy and France (at that time Cellogel was distributed by Sebia) found a higher proportion of small monoclonal components in patients with gammapathies than were found in the English and American centers. Clinical Chemistry, Vol. 37, No 11, pages 1917-1921. R.G. Jones, F. Aguzzi, J. Bienvenu, P. Bianchi, C. Gasparro, M. R. Bergami, A. Perinet, H. Bernon, G.M. Penn, I. Keller and J.T. Whicher.

To satisfy the needs of those who are convinced that the transparent gels, like agarose, are the best means for electrophoresis our company proposes Celloclear Agarose Plus, a transparent gel supported by mylar which beats the systems of commercial agarose for number of tests/hour aiming mainly at the diagnosis of gammapathies with methods of 54 test HRE/hour and IFE of 1,2,4,9,18 tests in 2 hours 30 minutes with greatly reduced manipulations.
3. THERAPEUTIC UP-DATING ON BLOOD DISEASES

The most important up-dating concerns the therapeutic successes in the treatment of myelomas and lymphomas sometimes accompanied by monoclonal bands, thanks to the new procedures of allogeneic transplants of staminal cells from the bone marrow and peripherical with the help of a new anti-rejection drug with zero toxicity in patients between 55 and 60 years of age (Prof. Corradini, Dipartimento di Ematologia Oncologica e Trapianti, Istituto Nazionale dei Tumori of Milan).

Also in the case of amyloidosis new treatments have been experimented with success in Pavia (Centro per lo Studio e la Cura delle Amiloidosi Sistemiche) while the research of Prof. G. Merlini and his collaborators proceed and are of great interest to the scientific community (it should be born in mind that amyloidosis AL is often initially noticed thanks to a small monoclonal band during electrophoresis, hence the importance of high resolution and the uselessness of miniaturised micro electrophoresis or of CZE). Recently Merlini and others confirmed the indispensability of immunofixation in high resolution for the diagnosis of amyloidosis AL (Biochimica Clinica, 2005, Vol.29, No 2, Page 240).

In the field of non-tumural diseases of the blood such as beta-thalassemia major or Cooley’s disease, Prof. Locatelli, Director of the department of Paediatric Onco-haematology, Policlinico S.Matteo, Pavia, and president of the AIEOP (Associazione Italiana Ematologia e Oncologia Pediatrica), in December 2004, communicated the successes and the optimisation of transfusional therapy and ferrochelating during the transplant of haemopoietic staminal cells extracted from umbilical cords. It is obvious that advances in this field of therapy must be accompanied by analytical diagnostic systems equally advanced such as high resolution electrophoresis on Cellogel in the case of immuno-proliferative diseases (myeloma, amyloidosis, etc) and valid electrophoresis of the hemoglobins on Cellogel alongside the work of the haematologist.
4. MANAGEMENT OF MONOCLONAL GAMMAPATHIES

Monoclonal gammapathies are asymptomatic immuno-proliferative diseases recognisable only by electrophoresis of the serum and/or urines of patients or, when it is too late, from an immunohistochemical test of a bone marrow biopsy. If the electrophoretic migration shows one or more bands of monoclonal immunoglobulins or monoclonal components produced by the proliferation of one or more B-cells of the lymphocytary system the patient is gammapathic. Electrophoresis of serum and of urine is the only irreplaceable method of discovering and diagnosing gammapathies, hence, only a valid method of electrophoresis makes a certain diagnosis possible.

For valid electrophoresis of the serum proteins the extension of the gamma zone must not be less than 20 mm so as to highlight even the smallest monoclonal band within it. This is characterised by its compact form which because of its clear definition can be seen even in a concentration of 1 g/L on a total gammaglobulins base with a densitometric value of 20% (bearing in mind that 1 g/L of a monoclonal band represents a tumoral mass of 10 g and is equal to 10 billion plasma-cells).

Having stated this, micro electrophoresis with only 0.3 μL of deposit, 20 mm migration and 7 mm extension of gamma zone, however attractive it may be to see, is not capable of revealing all the monoclonal gammapathies, because, within an extension of only 7 mm of gamma zone, it is impossible to see small or medium monoclonal bands; these, having only 0.3 μL deposit, are not even applied in the minimum quantity detectable by usual stains. In fact, according to the recommendations of the SIBioC commission on proteins in Italy and other reputed Italian and European authors on electrophoresis, micro electrophoresis should be avoided while the chosen method is the semi-micro elongated electrophoresis with 55 mm total migration and Amidoblack stain. Better still would be high resolution electrophoresis with 65 mm total migration and Coomassie BB 250 R stain, as in the methods proposed in this catalogue. Particularly, Cellogel HRE methods, have the same diagnostic validity as the “proposed selected method” of Jeppsson and Laurell when the purpose of electrophoresis is the diagnosis of gammapathies with the advantage of rapidity and ease of use and of their low cost. HRE on Cellogel assures the effective deposit of ng of the monoclonal band to be identified as well as a sufficiently high extension (over 20 mm) of the zone of the immunoglobulins and also the sensitivity to reveal small monoclonal bands (Coomassie BB 250 R stain).

High resolution electrophoresis on Cellogel or that on agarose according to Jeppsson and Laurell can reveal up to 98% of the monoclonal components; the semi-micro elongated on Cellogel can reveal 90% of the monoclonal components, while the micro-electrophoresis on agarose and on common cellulose acetate as well as Capillary Zone electrophoresis can miss over 50% of monoclonal components.

The Capillary is not able to analyse Crioglobulins or immunocomplexes in the serum and has difficulty in revealing the monoclonal IgM because it does not offer a sufficiently long gamma zone and because with a deposit inferior to 1 nanoliter no UV detector can see monoclonal bands with a concentration inferior to 1 g/L.
ASSESSMENT OF MONOCLONAL GAMMAPATHIES

a) High resolution electrophoresis of serum protein on Cellogel to reveal the presence of monoclonal components and thus to determine the number of patients with gammapathies, to be registered and monitored over time, including those with incipient immuno-proliferative diseases (Fig. 1).

![Fig. 1 - HRE semimicro on Cellogel](image1.png)

b) Immunofixation (IFE) of the serum of gammapathic patients with anti IgG, IgA, IgM, K and Lambda antisera for the classification and typing of the monoclonal components (Fig. 2). IFE with anti IgD or anti IgE when the precedent IFE of the serum showed only K or Lambda in the serum. Should the latter two not react this would signify the presence of a K free or Lambda free monoclonal in the serum, that is to say amyloidosis AL which can be confirmed with the Red Congo test on an umbilical biopsy and with IFE of the urine.

![Fig. 2 - IFE on Cellogel strips, 5.7x14 cm (reduced image)](image2.png)
c) IFE of the Bence-Jones in a gammapathic patient, according to the method proposed by the SIBioC (Biochimica Clinica, 2001, Vol. 25, No.1, pages 23-31) to observe the presence of real Bence-Jones protein, that is a K free or Lambda free monoclonal, and in some cases to reveal the ladders of pseudo Bence-Jones, that is polyclonal K or Lambda. The presence of a K free or Lambda free monoclonal in urine generally signifies the malignancy of the gammapathy (Fig. 3), while the absence of the Bence-Jones protein in the urine of a gammapathic patient gives an uncertain significance to the gammapathy or MGUS (Monoclonal Gammapathy of Uncertain Significance). The presence of an IgG, IgA or IgM monoclonal component in the IFE of urine with relative positivity of alligned K (bound) or Lambda (bound) can mean serious kidney damage as the kidney permits complete immunoglobulins to filter or may signify post-renal haemorrhage due to a broken capillaries in the bladder or urethra with the relative typical pattern of a gammapathic patient’s serum.

Once diagnosed, a gammapathic patient has to be monitored over time; according to the Scandinavian school high resolution electrophoresis should be repeated every 4-6 months for the first two years and once a year after this. Immunofixation should be repeated in the case that a quantitative increase of the monoclonal band (recommencement of the proliferation of the B-cell) be noted or if a second monoclonal band appears. In all cases the control and follow-up of a patient with a gammapathy should always be undertaken via high resolution electrophoresis. A patient with MGUS can be rapidly controlled with a simultaneous serum+urine IFE on Cellogel to remove any doubts considering that the presence of Bence-Jones in the urine generally signifies the malignancy of the gammapathy. In this situation it will be possible to anticipate the transplant, especially in the case of patients nearing 60 years of age.
Decisions about therapeutic intervention must also be made in the case of an increase of the intensity of the monoclonal component or the appearance of new monoclonal bands.
The quantitative nephelometric or turbidimetric immunochemical determination of IgG, IgA and IgM has no diagnostic value for discovering or for the follow-up a gammapathic patient; this type of determination cannot reveal, as opposed to electrophoresis, the monoclonality due to immuno-proliferation of the B-cell, producer of immunoglobulin characterised by the compactness of the small or large band, generally in the gamma zone, of the electrophoretic migration or sometimes in the beta or alpha 2 zone. The nephelometer or turbidimeter sum, for example, the polyclonal IgG and the monoclonal IgG giving a single number (concentration) without distinguishing between polyclonal or monoclonal IgGs. This concentration can be below the normal value or even much above the normal value without any possibility of indicating monoclonality. Even the ratio between K and Lambda which is normally 2:1, although it can be unbalanced in the case of the presence of a monoclonal component whose bonded chains are for example all K or all Lambda (as shown by the IFE), it is not sufficient to demonstrate the monoclonality of the immunoglobulins of the patient. High resolution electrophoresis and IFE are the only solution to guarantee the diagnosis of monoclonal and oligoclonal gammapathies.

In the conclusion of this chapter we do not in any way think of proposing solutions which could upset the convenient automatic procedures already adopted in the central laboratories of hospitals. We cannot, that is, suggest systems which would result in doubling the cases of monoclonal gammapathies which can be diagnosed in the everyday patient. We do, however, strongly recommend the adoption of simple and rapid systems of high resolution electrophoresis on Cellogel and on Celloclear Agarose Plus for the control of suspect samples; above all, if the laboratory is connected to the department of oncological haematology, it could reject the micro electrophoresis and CZE reports. The directors of institutes of haematology, who do not have a laboratory at their disposal, could be forced to have the reports repeated by the central laboratories requesting the more valid form of high resolution electrophoresis and also of high resolution immunofixation. These centres themselves, in the case of bone marrow transplants after chemotherapy, should not fail to analyse the donor with a simple preliminary high resolution electrophoresis which excludes the presence of monoclonal bands, that is immuno-proliferant B-cells, prior to performing the bone marrow biopsy for the immunoistochemical analysis of the same.

In order to train laboratory personnel specialised in the diagnostic techniques for gammapathies, our training service offers workshops and programmed demonstrations to rediscover monoclonal gammapathies adapting the laboratory interested to equal the excellence of the best few centres left for the study of gammapathies.
As we have already stated, the technology on agarose (miniaturised micro electrophoresis and the too manual immunofixation) and the automatic but limited Capillary electrophoresis have acquired a large portion of the market, lowering not only the quality and the diagnostic validity of clinical electrophoresis, but doubling, if not trebling public expenditure paid by hospitals for this very important analytical niche which concerns the diagnosis of diseases like myelomas, amyloidosis, thalassemiae, etc, and the search for proteins and enzymes in general.

In Italy in 1990 public expenditure, despite the introduction of Japanese automatic equipment, was estimated at about 20 billion Lire per year; today the total cost is over 35 million Euro (approx. 70 billion Lire) due to the high cost of semi-automatic electrophoresis on agarose and the Capillary system.

The advent of automatic, semi-automatic and Capillary equipment for electrophoresis has brought little time-saving of labour and no reduction of personnel assigned to electrophoretic analyses. The personnel assigned to this has lost their professional importance and any recognition of the technical quality of their work and reduced them to being little more than button pushers. This should cause both local health authority managers and the directors of hospital laboratories to reflect, especially where technical personnel of proven experience and technical capacity are present. In the few laboratories of excellence left in Italy known for their research on monoclonal gammapathies, given the same time employed and the same number of tests performed, not only is 70% less spent, but also the technicians assigned to electrophoresis receive their deserved professional satisfaction, and this is, of course, in the patients’ prime interests.
6. CELLOGEL: what is it and how is it used?

CELLOGEL

Cellogel is a film of cellulose acetate in gel form, produced in wet state to maintain its gel properties and facilitate the impregnation into the buffer solutions without the problem of air being trapped in its pores as can occur when using dry microporous acetate membranes. Cellogel is the ideal electrophoretic support for clinical electrophoresis and for the immunological techniques where it often out-performs agarose. Cellogel is an electrophoretic medium which separates the proteins, even at high resolution, according to the electric charge and does not have the effects of molecular filtration typical of other gels like polyacrylamide. Cellogel is packed in strips and sheets of various dimensions (Fig. 4).

If compared with German or American produced dry cellulose acetate (in microporous film for electrophoresis) Cellogel presents important properties and advantages:

a. Cellogel is ready for buffering and does not entrap air at the moment of immersion into the electrophoretic buffers.

b. In comparison with dry acetate, with a thickness from 120 to 160 microns, Cellogel is produced with thicknesses between 190 \( \mu \) up to 500 \( \mu \), depending on what it is to be used for. The greater the thickness, greater is the volume of the specimen which can be deposited on
it (for example with Cellogel with a thickness of 300 µ, a semi-micro applicator of 1.2 µl/9 mm can be utilised instead of the semi-micro of 0.9 µl/9 mm normally used with 200 µ Cellogel; this signifies that with specimens which are poor in proteins it is advisable to use a thicker Cellogel, thus depositing a larger quantity of proteins to be detected). Furthermore, higher thickness corresponds, with the same voltage applied during electrophoresis and with the same ionic strength of the buffer, to a higher passage of current measured in mA x strip (e.g. a 5.7x14 cm strip of 200 µ Cellogel impregnated with a buffer with ionic strength equal to 0.05 placed on a bridge 8.5 cm long in a 200 V electric field permits the passage of 5 mA per strip. If the same Cellogel were 250 µ thick, it would allow the passage of 6.5 mA per strip, if were 300 µ it would allow the passage of 7.5 mA and therefore (V x mA) more Watts).

c. With Cellogel there is the possibility to apply specimens with a volume of 0.9 µl/9 mm (semi-micro method) or of 2 µl/18 mm (macro method) without the sample spreading as would occur on a very thin dry acetate strip which tolerates micro applications of 0.25 µl/4 mm well but lets the semi-micro and macro deposits spread unacceptably. The application can be repeated two or three times on the same spot on Cellogel, when necessary, as in the case of electrophoresis of isoenzymes and of biological liquids poor in proteins.

d. Dry acetate is limited to the migrations of 20 mm of miniaturised micro electrophoresis or at most of 30 mm with a quasi-semi-micro carried out with stamp applicators and their relative dispocards. Cellogel, however, is suitable for standard migrations of semi-micro 35 mm serum proteins, with 45 mm semi-micro with prolonged migrations or high resolution electrophoresis with 60-70 mm migrations or more.

e. HRE (high resolution electrophoresis) is only possible on Cellogel and not on dry acetates.

HRE on Cellogel is much simpler and easier than on agarose; the expensive systems for the circulation of cold water or Peltier control which are needed for all the commercial agarose gels with a thickness of 500 microns are not required with Cellogel. HRE on Cellogel has a cost per test equal to a semi-micro test on acetate and does not have the prohibitive costs of agarose which is only produced in kits of 10 or maximum 15 tests per film, which cannot be proposed for the routine of large and medium size laboratories.

With French agarose it is only possible to carry out 10 tests/hour, with American agarose 15 tests/hour, while with Cellogel it is possible to perform up to 48 test/hour; furthermore HRE on agarose presents itself with migrations containing a floating β-lipoproteins fraction focused, sometimes, overlapped on a small monoclonal band. In practice, high resolution on agarose is a time consuming system as well as being defective.

Cellogel, like agarose, offers resolutions that depend on the length of the migrations. Making a deposit of 0.9 µl on a line 9 mm long and 1.5 mm wide (semi-micro deposit):

- After 35 mm movement of albumin the serum proteins migration shows 5-6 fractions
- After 50 mm it shows 7-9 fractions
- After 65 mm it shows 9-13 fractions
- After 110 mm it shows between 11 and 23 fractions
Chemically Cellogel is a film of water made of from 7-8% of solid cellulose acetate and 92-93% H₂O of which 60-70% is constitution H₂O bound with hydrogen bridges, and 20-30% water for impregnation of the pores. The evaporation and water transport onto the membrane during prolonged electrophoresis is better regulated, the evaporation of the constitution water bound by the hydrogen bridge is much slowed down and this facilitates long migrations which are impossible on dry acetate. The porosity of Cellogel is predisposed for the main analysis, that is electrophoresis of the serum proteins. Large molecules like pre-β-lipoproteins and all the other serum proteins penetrate and migrate. Only the chylomicrons do not penetrate or migrate and only leave a mark at the start point, the same occurs with immunocomplexes and cryoglobulins when present; these marks which are analytically and diagnostically important, cannot be seen on the French agarose which uses filtering applicators. The predisposed porosity of Cellogel is decisive in avoiding spreading of samples at the moment of depositing and spreading of the fractions with low mobility during migrations which can be lengthy. All in all the right porosity corrects the insufficiencies of other commercial cellulose acetates membranes. To this must be added the better compatibility between Cellogel and serum proteins, including lipoproteins, that are incompatible with agarose. The latter is, in fact, a film of water (99% H₂O) totally hydrophilic, where the amphiphilic serum proteins with more lipophilic characteristics remain floating on the surface even when the sample is deposited with applicators which cut the gel. The superiority of Cellogel over agarose was recognised in numerous publications by important authors between 1963 and 1971. Thanks to its amphiphilic properties (hydrophilic and lipophilic) Cellogel has optimal compatibility with specimens as difficult and complex as serum proteins, which are also amphiphilic. Cellogel is, therefore, the ideal support for electrophoresis of serum proteins, hemoglobins, lipoproteins, isoenzymes, for all the immuno-electrophoretic techniques and for the search for antigens, antibodies and tumour markers (especially those immunofixable with polyclonal antibodies).

THE USE OF CELLOGEL

Conservation of the strips

Cellogel in its original packets can be preserved for an unlimited period, it resists at temperatures from -10°C to +40°C and does not have the problems of agarose in tropical climates. After opening the packet it is important that the strips are not left exposed to air and consequent drying and loss of the properties of the gel with wrinkling and destruction of the micro-porous structures. The strips must be preserved in a solution of 30% methanol in water, in its packet or in a covered plastic box containing about 100 ml of methanol solution.

Buffering of the Strips

To properly impregnate the strips in the buffer, a minimum of 10 minutes shaking is recommended. When many strips are immersed in the buffer, agitate manually or better with a rotating shaker (Fig. 5) to avoid the strips forming a compact sandwich. Do not leave for hours or for days the Cellogel immersed in alkaline buffer because basic buffers, used for serum proteins after prolonged immersion could induce de-acetylation of the cellulose acetate. The most commonly used buffer for serum electrophoresis on a semi-micro or micro scale is Tris-Hippurate, pH 8.8 and
ionic strength 0.05. The Tris-Hippurate substitutes the barbiturate buffer Veronal-Tris, pH 8.8 and ionic strength 0.05, used before the laws which assimilated barbiturates to drug narcotics.

Preparation of the Electrophoretic Tank

Completely fill one compartment of the tank with the buffer solution. Tilt the tank (approx. 30°) to level the buffer in the two compartments. (Fig. 6).

Preparation of the specimens

Semi-micro electrophoresis: pipette 30 µl of each sample on the first row of the 4x drop-holder of the base of the specimen holder (Fig. 7) then continue with the second row and the third row if the total samples are 12. With the semi-micro method samples with a dilution of 1:3 are required, pipette 20 µl of buffer or saline solution on each position of the drop holder and then pipette 10 µl of serum stirring it with the tip of the pipette.

Micro electrophoresis: pipette 20 µl of each sample on the first row of the 8x drop-holder of the base of the specimen holder (Fig. 7), continue with the second and the third row if the samples are 24.
Blotting Cellogel, identification of the penetrable surface and positioning of the strips on the bridge

Extract the strips from the buffer solution and place them on a sheet of filter paper with the penetrable surface uppermost (Fig. 8a), that is, with the cut angle on the lower right-hand side. Remove the excess buffer solution with a second sheet of filter paper and place the strips on the bridges, maintaining the penetrable surfaces facing upwards. The strips must be well-stretched and held with the apposite plastic clips (Fig. 8b).

If a tank manufactured by another company is used in which paper wicks are utilised, these should be washed in the buffer solution for the same length of time as that used to impregnate the Cellogel strips.
Application of the samples and electrophoretic migration of the sera

Load the tips of the applicator (semi-micro or micro) taking the samples from the first row (Fig. 9a). Place the applicator on the first bridge (selecting the first notch, for example, if the deposit is to be at 27 mm distance from the negative border) and then press the lever of the applicator with a finger. This will lower the tips until they touch the surface of the Cellogel (Fig. 9b). Leave the tips lowered for at least 10 seconds if the whole sample is to be taken up. Make sure that the depositing is at the negative pole. Wash the tips twice in the water at the base of sample holder and unload it onto a sheet of filter paper placed on the table.

Reload the applicator with the specimens from the second row and deposit them on the strip on the second bridge. Re-wash the tips, load and apply the specimens from the third row on strip 3. Number progressively, with a black biro pen (avoid blue or red), the three strips for their identification. Cover the electrophoretic tank with its lid and make sure that the jacks are correctly inserted in their respective sockets (red for the positive pole, black for the negative). Switch on the power supply, apply the voltage required by the method (e.g. 200 V) and regulate the time of migration with the timer (e.g. 35 minutes for semi-micro or 22 minutes for micro).

![Fig. 9a - Applicator loading](image)

![Fig. 9b - Samples application](image)
Observe the milliammeter

If much more than the initial 5 mA is indicated for a 5.7x14 cm strip (15 mA for 3 strips per 1 tank) it is probable that there is a short circuit (for example, a link between the positive and the negative compartments) or an erroneous dilution of the buffer solution.

If zero is indicated, this means that no current passes through the tank because the lid has not been correctly positioned (the magnets do not turn on the security micro-switches), or a jack has not been properly inserted, or an electrode has broken, (in this case contact the assistance service).

Staining

At the end of the migration time, switch off the power supply, extract the first bridge (taking care not to drip on the other strips) and proceed with the specific staining.

Specific stains

Electrophoretic analysis of serum, as opposed to analysis with Capillary Zone Electrophoresis, is rendered specific by treating the strips, on which migration of the sera occurred, with specific stains.

Staining of the Serum Proteins, semi-micro and micro. The most commonly used staining solution is Ponceau S (code 03C02-S1L). Immerse the strips in the staining for 5-7 minutes and recuperate it after use. Destain the strips with three rinses of destaining solution (5% acetic acid or 3% citric acid). If a better contrast is desired Amidoblack (code 03C01-SB) can be utilised and the relative destaining solution (475 ml methanol + 475 ml distilled water + 50 ml glacial acetic acid).

Staining of Lipoproteins. The specific stain used is Sudan Black B (code 03A04-P). Immerse the strips in the staining solution for approx. 45 minutes. Wash delicately under running water.

Staining of Glycoproteins. Use the Schiff reagent, see page. 45.

Staining of Isoenzymes. After migration dozens of different isoenzymes are found fractioned on the strip of Cellogel together with the serum protein. Staining each type of enzyme is realised with specific substrate chromogens. For example, if lactate dehydrogenase (LDH) is to be identified in the migrated serum it is necessary to stratify on the strip, while it is still stretched on the bridge, about 100 μL of a specific substrate chromogen for LDH (code 08C01) with the volumetric distributor. Transfer the bridge with the strip to a damp box and incubate at 37° C for 30 minutes until the violet stain of the Fenazine Metasulfate + MTT develops.

Staining of individual immunofixated proteins. Immediately after migration the single proteins can be immunofixed stratifying a specific antiserum by means of the volumetric distributor. The strip on the bridge is left to incubate for 15 minutes, then it is washed with saline solution to remove all the proteins that have not reacted with the antiserum and finally stained with Amidoblack or for
an higher sensitivity with Coomassie. This operation can also be carried out with immunofixation by means of peroxidase marked polyclonal antibodies (if available) if the object of research is a trace of protein which requires highly sensitive revelation (100 picograms/band sensitivity), e.g. tumour marker or IgG of cerebrospinal fluid. After incubation and washing in TBS or PBS the revelation occurs with a chromogen of the peroxidase (AEC+DMFA+Acetate buffer+ H₂O₂) code 890 MA.
In all cases follow the detailed protocols for the relevant methods of Cellogel electrophoresis. This paragraph of specific staining shows the great potentiality of Cellogel in the research of the many components of serum and takes for granted the incomparability of this highly specific technology with those aspecific technologies such as Capillary Zone Electrophoresis.

The transparency of Cellogel

Immerse destained Cellogel strip in the clearing solution (code 06A06-S1) for 30 seconds. Place the strip on a film of mylar (code 13M01-100) or on a glass slide and remove the excess solution or possible air bubbles with a glass rod or with a roller. Dry the strip in an oven at 80°C for 10 minutes.
Clearing on a film of mylar is preferable to that on a glass slide because Cellogel coupled with mylar cools in not more than one minute.

Quantitative Densitometry

The cleared film, when cooled, is ready for densitometry with a 520 nm photo-densitometer in the case of fractions stained with Ponceau S or with Glob AI Scan software (see page 29) using, for example, the semi-micro red serum proteins programme.

*Hundreds of publications about Cellogel are available at www.pubmed.gov*
7. WHAT TO ORDER

The normal equipment for routine clinical and/or research electrophoresis is the following:

- Power supply 0-300V (code 10A08/B) two outputs for two electrophoretic chambers.

- 1 chamber (code 11A11-CE) with three France bridges for 5.7x14 cm or 2.5x14 cm strips. 1 long bridge for 18.3x14 cm and 14x14 cm sheets suitable also for use with three 5.7x14 cm strips and six 2.5x14 cm strips. Should more than 24 micro tests or more than 12 semi-micro tests need to be carried out, order a second chamber which permits the doubling of the number of tests.

- 1 micro applicator (code 12A08/8P2) for 8 samples of 0.3 μl/5 mm.

- 1 semi-micro applicator (code 12A08/4P4) for 4 samples of 0.9 μl/9 mm or 1 semi-micro applicator (code 12A05) for 4 samples of 1.2 μl/9 mm (recommended for electrophoresis of hemoglobin and isoenzymes).

- 1 semi-micro applicator (code 12A02/SU) of 0.7 μl/7 mm for simultaneous IFE of serum and urine.

- 1 semi-micro applicator (code 12A20/18P2) for 18 simultaneous aligned deposits of 0.7 μl/7 mm for sheets measuring 18.3x14 cm and 18.3x17 cm.

- 1 densitometer Glob-Al Scan, automatic, universal (code 18A18/1) made up of software compatible with the customers computer having an operative Windows 98, 2000 or XP system and scanner. For the simultaneous reading of from 1 to 144 samples, for the filing of thousands of reports with colour images of the migrations and the transmission of data to the host computer.

- The use of a rotating shaker (code 13A34) is recommended for the buffering, staining, destaining, rinsing in saline solution etc. in the plastic boxes complete with lid (code 13A50-13A53).
8. EQUIPMENT FOR ELECTROPHORESIS
ON CELLOGEL

POWER SUPPLY

**Code 10A08/B**
The Power Pack 300 V is suited to all types of electrophoresis requiring up to 300 V. The digital display indicates the voltage values (which can be constantly regulated) and the current. With two outputs, 2 tanks can be supplied at the same time.

**Voltage:** 0-300 V DC current  
**Amperage:** 0-100 mA  
**Exit Points:** 2 (for 2 tanks)  
**Timer:** 3s - 60h  
**Input:** 220V (on request 110 V) 50/60 Hz  
**Dimensions:** 27x21x11 cm  
**Weight:** 3.8 Kg

ELECTROPHORETIC TANK FOR CELLOGEL

**Code 11A11-CE**
Tank for electrophoresis on Cellogel and cellulose acetate in general. Designed for routine and research needs.

Functions with six strips 2.5x14 cm or with three strips 5.7x14 cm on three bridges, model France of 8.5 cm, furthermore it works with the same size strips as above and with sheets 14x14 cm or 18.3x14 cm on an 8.5 cm long bridge with plastic clips. The tank and the bridges are injection moulded in polycarbonate with high chemical and physical resistance. The lid is in smoky grey semi-transparent polycarbonate with two magnets which work safety micro-switches and cut off the current when the lid is taken off.

**Electrodes:** Platinum, 7 cm  
**Dimensions:** base 25x19 cm, height 5 cm  
**Capacity:** 200 ml of buffer soln. per compartment  
**Weight (including the 4 bridges):** 1 Kg
BRIDGES FOR CELLOGEL TANK

a - Cod. 11B06-1
France bridge, 8.5 cm, for strips of 2.5x14 or 5.7x14 cm.

b - Cod. 11B03
long bridge for sheets of 18.3x14 or 14x14 cm and strips of 2.5x14 or 5.7x14 cm. Migration field 8.5 cm.

c - Cod. 11B15-1
France bridge, 11 cm, for strips of 2.5x17 or 5.7x17 cm, Rectangular.

d - Cod. 11B04
long bridge for sheets of 17x17 cm and strips of 5.7x17 cm. Migration field 11 cm.

e - Cod. 11B14
France bridge, 14 cm, for Cellogel RS Wedge of 5x18.5 and 5.7x18.5 cm Rectangular.

APPLICATORS FOR CELLOGEL

Micro 8P2 code 12A08/8P2
Can be clipped onto all France bridges. It applies 8 specimens simultaneously to the Cellogel strip of 5.7 cm width, leaving 8 marks of the same dimensions as Figure 10a. Each of the 8 tips applies 0.3 µl on 5 mm. The unloading of the tips is complete after the application time of at least 10 seconds. The specimen holder base has 3 rows of 8 drop holders onto which volumes of 20 µl serum are pipetted. This applicator enables the rapid application of 24 samples on 3 strips already positioned in the chamber. If the first notch is
used the deposits are aligned at 27 mm distance from the cathodic edge. If the last notch is used the deposits are aligned 9 mm from the cathodic edge. The applicator serves not only for classical micro electrophoresis with 20 mm migration but also for the “Microlong”, i.e. high resolution micro-electrophoresis described on page 36. This latter method is recommendable for the rapid search for monoclonal gammapathies including incipient ones.

**Semimicro 2+2 code 12A02/SU**

Studied for simultaneous IFE of serum and urine (kit Code 08C09). It can be clipped onto France bridges. It applies 4 specimens simultaneously on two strips, 2.5x14 cm, positioned at the lateral extremities of the bridge. Each of the 4 tips applies 0.7 μl on 7 mm. The unloading of the tips is complete after an application time of at least 10 seconds. For this type of use 25 μl of serum and 25 μl of concentrated urine are pipetted onto the drop holder of the base of the sample holder.

It can be utilised for semi-micro electrophoresis of 2 samples on strips 2.5x14 or 2.5x17 cm.

**Semimicro 4P4 code 12A08/4P4**

It can be clipped on all France bridges. It applies 4 specimens simultaneously on the 5.7 cm wide Cellogel strip leaving 4 marks of the same dimensions as the figure 10c. Each of the 4 tips applies 0.9 μl on 9 mm. The unloading of the tips is completed after an application time of at least 10 seconds. The base of the specimen holder has 3 rows of 4 drop holders onto which 30 μl are pipetted. This applicator makes it possible to deposit 12 samples rapidly on three strips already positioned in the tank. If the first notch is used the deposits will be aligned at 27 cm from the cathodic edge. If the last notch is selected the deposits will be aligned 9 mm from the cathodic edge.

This applicator serves not only for classical semi-micro electrophoresis with 35 mm run but also for the prolonged semi-micro of 45-55 mm.
Semimicro 4P code 12A05
Recommended for electrophoresis of hemoglobins.
It can be clipped onto all France bridges and applies 4 specimens simultaneously onto the 5.7 cm wide Cellogel strips leaving marks of the same dimensions as figure 10d. Each of the tips applies 1.2 μl on 9 mm. The unloading of the tips is completed after an application time of at least 10 seconds. The base of the specimen holder has 3 rows of 4 drop holders onto which 30 μl serum are pipetted. This applicator renders it possible to deposit 12 specimens rapidly on three strips already positioned in the tank. If the first notch on the bridge is selected, the deposits will be aligned at 27 mm from the cathodic edge. If the last notch is selected the deposits will be aligned at 9 mm from the cathodic edge. This applicator is utilised with Cellogel 250 μ or more thick, for deposits, even repeated, of serum to determine the lipoproteins or the isoenzymes (e.g. for the gamma GT which needs 4 repeated deposits for a total of about 5 μl).
This applicator cannot be used on dry micro-porous acetate membranes because the deposit would spread giving an unacceptable start point for good electrophoresis. The deposit, even repeated, on Cellogel maintains a perfect mark and is a suitable start point for excellent migration.

Semimicro 6P2 code 12A08/6P2
It can be clipped onto all France bridges and applies 6 specimens simultaneously on the 5.7 cm wide Cellogel strip leaving 6 marks of the same dimensions as figure 10b. Each of the 6 tips applies 0.7 μl on 7 mm. The unloading of the tips is completed after an application time of at least 10 seconds. The base of the specimen holder has 3 rows of 6 drop holders onto which 25 μl serum are pipetted. This applicator renders it possible to deposit 18 samples rapidly on three strips already positioned in the tank. If the first notch of the bridge is selected the deposits will be aligned at 27 mm from the cathodic edge. If the last notch is selected the deposits will be aligned at 9 mm from the cathodic edge.
**Semimicro 18P2 code 12A20/18P2**

It can be clipped onto long bridges (code 11B06 and code 11B15).

It applies 18 specimens simultaneously on Cellogel sheets positioned on the above mentioned bridges. Each of the 18 tips applies 0.7 µl on 7 mm. The unloading of the tips is completed after an application time of at least 10 seconds. The disposable specimen holder is moulded in transparent polystyrene and is inserted into the base of the applicator. It holds 3 rows of 18 rectangular sample points, 9 mm pitch, suitable for multiple pipetting dispensing 25 µl of specimens. This applicator can be used for high resolution electrophoresis of 18-36 samples with two tanks. (see method on page 37).

**Applicator Box, micro, semi-micro and macro, code 12A07**

This set is suitable for single applications.

The box contains:
- 1 Applicator micro 0.3 µl/5 mm
- 1 Applicator semi-micro 1.2 µl/9 mm
- 1 Applicator macro 2 µl/15 mm
- 1 Serum holder base for 10 micro-semimicro and 6 macro
- 1 Applicator holder with a lever to lower the tip until contact with the surface of Cellogel.

**ACCESSORIES**

**Guide ruler for Applicator Box, code 11B11**

This accessory of the applicator box can be placed on the edge of the tank so that the distance between the various deposits can be selected.
Guide ruler for Applicator Box, code 11B13
This is an important accessory for the applicator box. The ruler can be fastened onto the bridges, codes 11B03 and 11B04.

Volumetric Distributor, Model DC/3, code 13A23
With this device, moulded in transparent polystyrene, it is possible to distribute on the surface of Cellogel strips, 2.5 cm wide, laid on the electrophoretic bridge, measured volumes of 40, 75 and 100 µl of anti-serum (immunofixation) or an enzymatic substrate (isoenzymes LDH, CPK, Gamma GT, etc) obtaining a large saving in reagents which generally have high costs. Particularly, in the case of the IFE of 1 patient on a semi-micro scale after electrophoresis of serum+urine on 6 strips (Kit code 08C09), the first strip is placed in staining while the second is stratified with the DC/3 Volumetric Distributor with 40 µl of anti IgG anti-serum, 40 mm long in the migration zone. The same is undertaken with the 3rd, 4th, 5th and 6th strips respectively with anti-IgA, anti-IgM, anti-K and anti-Lambda antisera. The five distributors are washed while the 5 strips are left in incubation at room temperature for 15 minutes. At the end of incubation, the strips are washed in saline solution and they are stained with amidoblock (see instructions for use of Kit, code 08C09). Another important use of the Volumetric Distributor is the stratification of the stain substrate to reveal, for example, the LDH isoenzymes of the serum, immediately after the electrophoretic migration of the samples. In this case are sufficient 75 µl of the expensive reagent of NAD, MTT and Fenazine Metasulfate stratified on the surface of 2.5x14 cm strips still laid on the bridge to reveal the 5 isoenzymatic fractions of LDH after 30 minutes incubation at 37°C (see instructions for use of staining solution, code 08C01).
Volumetric Distributor, Model DC/6, code 13A24
This device is used to distribute volumes of 100-150 μl of antisera or enzymatic chromogens on 5.7x14 cm strips laid on the France bridge of the electrophoretic chamber. The distributor DC/6 is ideal for preparing the Cellogel strip impregnated with 2-4 μl/cm² of specific anti-serum to determine the quantity of single proteins by the Laurell technique (Rockets). See Electroimmunodiffusion instructions for determination of albumin, alfa1 anti-trypsin, anti-Lpa etc. The DC/6 model is also used for two dimensional immunoelectrophoresis to stratify, for example, 140 μl of total anti human antiserum to perform the second dimension consisting of the migration in antibody field of all the human proteins separated during electrophoresis of the first dimension.

Plastic Guide for two-dimensional Immunoelectrophoresis, code 13A26
The guide is clipped onto the 8.5 cm bridge on which the 14x14 cm sheet of Cellogel has been placed and is used to deposit the serum diluted 1:4 of the first dimension and to guide the DC/6 distributor in the second dimension at the moment of applying the antiserum. Cellogel is unique for the performance of this technique which is also used by producers of antisera to control their specificity. 2D-IEP on Cellogel offers a higher sensitivity to Agarose and exceptional simplicity because the sample containing immunoglobulins does not retromigrate and does not need preliminary carbamilation to chemically modify the immunoglobins as required by Agarose.

Plastic Guide for Crossover, code 13A29
It can be clipped onto France bridges. This guide, moulded in transparent polystyrene, permits the depositing of sera, anti-sera or pure antigens in the Crossover technique or counter-immunoelectrophoresis on buffered Cellogel 5.7x14 cm placed on the bridge.

Guide for quantitative Radial Immunodiffusion (Mancini Technique)
This guide is needed only for depositing specimens on “Cellogel/Myl” 6x7.6 cm (Cellogel on a film of Mylar) and impregnated with 1-2 μl of antiserum per cm². To perform this quantitative technique at very low cost it is necessary to master the use of Microcaps of 1 μl required for a perfect deposit of the samples, generally diluted and pre-stained with Bromophenol Blue.
Transparent box
Plastic box for buffering, staining, destaining, washing, etc. processes.

Mini Transilluminator, code 13A16
The Transilluminator is used for optical intensification of the fractions on dried and whitened Cellogel, the revelation of proteic components up to 20 ng/band and photographic recording of the migrations.

Rotating shaker, code 13A34
This apparatus, simple and strongly built is intended for buffering, staining, destaining, washing in saline solution, etc. The shaker functions at the speed of 60 rpm and is particularly suited to and indispensable for electrophoresis and immunotechniques on Cellogel, such as IFE, Laurell technique, two-dimensional immunoelectrophoresis, etc. See Fig. 5 on page 14.

Mylar Film, code 13M01-100
Mylar films, 5.7x10 cm, are recommended in place of glass slides as a support for transparency of Cellogel. This, if the clearing solution (code 06A06-S1) is used, sticks to the support forming a single film. Compared to the procedure on glass, the coupling of Cellogel on Mylar, once extracted from the oven at 80°C, cools in 1 minute and is immediately ready for densitometry.

Mylar Film, code 13M04-100
The Mylar films, 18.3x14 cm, are used to form a sandwich of wet Cellogel with a pattern to photocopy straight after destaining procedure.
Automated Processor
This new automatic apparatus will be available in the autumn of 2006 and will permit programmed washes. The high speed peristaltic pump automates the buffering, staining, destaining, washing, etc. processes. The rocking container of the films in a horizontal position reduces the consumption of reagents to a minimum.
9. UNIVERSAL DENSITOMETER
GLOB-AL SCAN

In 1997 densitometry of electrophoretic migrations, on strips and sheets of Cellogel, on cellulose acetate supported or not, and also on agarose film, made an extraordinary step forward with the introduction of the first Glob-Al Scan; the electro-mechanical densitometers were capable of reading 8 micro electrophoresis, 4 semi-micro, 16 semi-micro simultaneously on sheets (multipolar) or one single micro or semi-micro migration on small strips. All these densitometers have been replaced by software technology, scanners, readers of optical density of micro, semi-micro and macro electrophoresis arranged on the normal strips or even on sheets containing up to 144 micro migrations. Glob-Al Scan software, installed on the client’s computer with Windows 98, 2000 or XP, and printer and scanner suggested by us, is capable of reading all types and formats of electrophoresis which fit onto the scanner surface in one single scan. Placing the frames relative to one’s own electrophoretic programme one obtains: a reading of the optical density of all the migrations, densitometric curve of each single migration, the quantitative data in absolute values and in percentages of the protein fractions, the ratio albumin globulin and the total protein value. The Glob-Al Scan reads migrations in different colours (e.g. Red, Blue or Green) and regulates filtration so as to obtain a reading of the maximum optic density (maximum absorption); once set on one’s own programme, Glob-Al Scan reproduces the same conditions for quick reading of successive electrophoretic migrations.

The reading of optical density is obtained with the scanner regulated at 72 dpi, value equivalent to photocells of classical densitometers for electrophoresis. The programme performs a complex calculation which mathematically transforms the data of reflection and reflectivity into optical absorption expressed in the usual logarithmic scale from 0 to infinity O.D.

Glob-Al Scan contains the classic programmes for serum proteins, hemoglobins, high resolution, lipoproteins, isoenzymes and for recording immunofixation (from 1 to 18 tests) with typical IFE images of 1 patient.

Memory capacity for over 100,000 reports, complete with images and curves.
Colour printing of reports on A4 paper.
Glob-Al Scan software contains the protocol for the transfer of data to the host computer. A bar-code gun accessory makes it possible to read the test tubes with a bar-code thus drastically reducing the time needed for reporting patients’ results.

The software is available in Italian and English.

Code 18A18/1 Software on CD Rom, protection key, licence and instruction manual.

Code 18A18/2 Flatbed Scanner.

Code 18A18/3 Bar-code gun.
Serum proteins report on Glob Al Scan

<table>
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<tr>
<th>Fraction</th>
<th>%</th>
<th>% Normal</th>
<th>g/dL Normal</th>
<th>g/l Normal</th>
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<td>53.68</td>
<td>4.39</td>
<td>3.70-4.90</td>
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<td>2.6</td>
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<td>0.14-0.35</td>
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<td>8-13</td>
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<td>0.50-0.90</td>
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<tr>
<td>Gamma</td>
<td>10.7</td>
<td>12-19</td>
<td>0.74</td>
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</tbody>
</table>

Total Proteins: 6.90 g/dL
AVG Ratio: 1.75

Patient name: Frank Anderson
Patient ID: 38766
Birth date: 26
Department: 09/04/2005
Access number: 26
Request date: 09/04/2005
BUFFERS

The first buffer solution for electrophoresis of serum proteins on Cellogel was the same one used for electrophoresis on paper, that is, Veronal sodium, pH 8.6, ionic strength 0.05, made up of diethylbarbituric acid and diethylbarbiturate of sodium. Later this buffer was modified in the research laboratory of Chemetron into a multi-fractioning or high resolution buffer with the addition of 7.20 g/L of Tris (hydroxymethyl) aminomethane. The Tris is an organic cation, which in an electric field, moves from the positive pole towards the negative with a cloud of 70 molecules of H₂O creating, in counter-current to the protein migration, a flow of “water transport” which is much greater than that determined by the Na⁺ ions which travel surrounded by a cloud of only 14 molecules of water.

The publication of the formula of the Veronal-Tris buffer, included in the instructions for use of Cellogel, was fortunate for users of competitive membranes of dry acetate who adopted Veronal-Tris to improve their micro electrophoresis thanks to the greater hydration offered by Tris on these thin membranes during electrophoresis. At the end of the 70s barbiturate buffers were considered to be drug narcotics by American, Italian and other European legislations. The necessity, therefore, arose both for the producers and for the users to turn to non-barbituric buffers for electrophoresis to avoid the burocratic problems involved in authorisations, registrations, etc. The research for alternative buffers resulted in a buffer which is even better than Veronal, the Tris-Hippurate, pH 8.8, ionic strength 0.05.

For over 20 years Tris-Hippurate has substituted Veronal not only for the superimposability of the results of separation in the serum proteins but also for the greater possibility of use in electrophoretic techniques, as in the case of certain enzymes, where Veronal was not satisfactory. The Tris-Hippurate is supplied to meet the most varied needs of customers:
- in powder in 10 bags for 10 litres of solution. Code 02A13-10
- concentrated in one 500 ml bottle for 5 litres of solution. Code 02C13-2X-5
- concentrated in 6x100 ml bottles for 6 litres of solution. Code 02C13-2X-6

A version of Tris-Hippurate, for laboratories for which the separation of β₁-β₂ (transferrin-C₃) is undesirable, is supplied in concentrated form of 6 bottles of 100 ml for 6 litres of solution. Code 02C13-2X-6 Na.

Another important buffer, proposed in international scientific literature by the Chemetron research laboratories, is the Tris-Glycine, pH 9 (code 02A01-10), characterised by high molarity and by low ionic strength (approx. 0.025). Multi-fractioning buffer, capable of compensating the high salinity of electrophoresis of specimen of unconcentrated urine deposited in the order of 50-100 μl in drop form on Cellogel RS Wedge (see page 49). This buffer has resolved the separation of hemoglobin better than Veronal. The procedure relative to hemoglobin with Tris-Glycine is described on page 41 and is a standard international method for the quantitative determination of HbA₂ and the correct diagnosis of Thalassemia minor.

The Tris-Glycine pH 9 (code 02A01-10) is only supplied in powder form in packets of 10 bags for
15 litres of solution.

**TGS Buffer.** The addition of Salicylic acid to the Tris-Glycine buffer improves high resolution. Salicylic acid is added to the albumin increasing its mobility and thus favouring the separation of Alfa1 and possibly its splitting into two variants. Of particular importance is the length of migration and retromigration of the Gamma zone with the maximum amplification of the possibility to discover small monoclonal bands. This buffer is used in high resolution electrophoresis (Microlong HRE and semi-micro HRE) for the diagnosis of monoclonal gammapathies. The TGS (code 02A03-10) is only supplied in powder form in packets of 10 bags for 10 litres of solution.

In the price list the specific buffers are described, for example **Tris Tricine** (code 02C01-6) for the separation of Gamma GT isoenzymes, for high resolution of the serum proteins or for semi-micro electrophoresis without retromigration of the gammaglobulins.

The guarantee covering Cellogel is not valid if the Customer uses non-original reagents.

**STAINING AND DESTAING SOLUTIONS**

**Ponceau S, Amidoblack, Coomassie** and **Sudan Black** stains are supplied in powder form, in concentrated solutions and ready for use. For automatic equipment, for example, the Price List proposes the solution of Ponceau S concentrate in a 1 litre bottle for 6 litres of solution (or 100 ml + 500 ml H₂O).

The destaining are the following: destaining No. 1 with 5% acetic acid for Ponceau S, **Ecological destaining** with odourless citric acid and destaining No. 2 for Coomassie and Amidoblack.

**Super Gold Stain** (code 23G05) for 5 fresh preparations of colloidal nucleated gold stain, 30 nm diameter, for staining of at least 80 unconcentrated urinary proteins after separation at high resolution on Cellogel.

**Silver Blue Stain** (code 08C36-2) for 2 operations of 16/32 tests of unconcentrated urinary proteins or for unconcentrated cerebrospinal fluid. The Silver Blue Stain kit for cellulose acetate was discovered by the Institute of Oncology, Bratislav, and is the only Silver stain for cellulose acetate existing on the international market. The staining of the bands at the end of the treatment makes the brown turn into brilliant blue after a bath of few seconds in the reactive blue toner (included in the kit) giving indelible conservation of the migrations. The Silver Blue Stain makes it possible to establish the typical patterns of selective or non-selective glomerular proteinuria, tubular proteinuria, mixed proteinuria, renal and post-renal, myelomatose proteinuria of Bence-Jones (K-free or Lambda-free). The sensitivity is at least two times superior to that of Gold Stain (See method on page 38).
ANTISERA FOR IMMUNOFIXATION

High avidity goat antisera, stained blue, for fast incubation of only 10-15 minutes, are available in packets of 1 ml each. They are included in all IFE kits.

CHROMOGEN SUBSTRATES FOR ISOENZYMES

A laboratory using Cellogel which already has available the strips and buffers needing to perform analyses of isoenzymes like Lactate Dehydrogenase (LDH), Alcaline Phophatase (AP) and the Gamma GT, can request the specific staining substrates. Substrates not included on the Price List can be supplied on request.

CLEARING SOLUTION

The clearing solution (code 06A06-S1) with a diacetone alcohol base contains an additive which facilitates the adhesion of Cellogel to Mylar films. Cellogel strip, after a bath in the clearing solution for 1 minute, can be placed into a oven (70-80°C) for 10 minutes. The transparent film on Mylar is immediately ready for densitometry without wasting time for cooling as occurs with clearing on glass. It is supplied ready for use in a packet of 6x200 ml bottles.
DRYING WHITENING SOLUTION

The whitener (code 07A02-S) is used to transform Cellogel into dry micro porous film without the problems of wrinkling of the film due to loss of gelatinization.

It is sufficient to immerse the perfectly destained Cellogel strips into the whitening solution for 3 minutes, place the strips on the glass plate or a Mylar film, dry the excess liquid with filter paper and leave them to dry at room temperature.

With the whitening and drying process the microfractions of the migrations, which would be invisible observing the wet strips, are enhanced and can be photographed by placing the strip on the transilluminator, possibly in a dark room. Whitened Cellogel is the most sensitive electrophoretic means if compared to transparent gels, such as agarose and polyacrylamide and with dry cellulose acetates.

READY-TO-USE KITS

An obvious advantage of these kits is the standardization of electrophoresis techniques and the guarantee of excellent end results. Every kit is supplied with detailed instructions for use of each phase of electrophoretic analysis. The use of these kits offers certain savings for the laboratory, in fact, the cost per kit includes all the material necessary. The expiry date of the kits varies from 1 to 2 years. Kits are available for micro and semi-micro electrophoresis of the Serum Proteins, Microlong HRE and semi-micro HRE, semi-micro Lipoproteins, semi-micro Hemoglobin, HbA1C Glycosylate Hemoglobin, Acid Hemoglobin, Immunofixation for 1 patient of serum-urine and for 6-8 patients.
A company operating internationally with over 40 years experience and technical know-how on clinical electrophoresis could not back out of satisfying the request from the market for “micro electrophoresis”. Consequently the Celloclear Agarose Plus catalogue presents the system for micro electrophoresis of 72 samples on 18x12 cm sheet and 144 samples on an 18x24 cm sheet within one hour, improving on what French and American competitors propose.

The present programme of electrophoresis on Cellogel intends to fill the analytical gap present on the electrophoresis market overcoming the incapacity of Capillary Electrophoresis and of miniaturised micro electrophoresis to supply a satisfactory diagnostic answer to patients undergoing electrophoresis of serum proteins when looking for monoclonal gammapathies.

It is, in fact, not admissible that methods persist and maintain their undeserved success, methods which are not able to reveal more than 50% of the monoclonal components.

With Cellogel we wish to fill the gap due to the lack of high resolution electrophoresis automatic and semi-automatic systems on the market with an acceptable routine method which assures that a large number of asymptomatic patients undergo analyses capable of establishing the presence of an immunoproliferation in act even if of small or medium significance.

In reality, high resolution electrophoresis on Cellogel can show not more than 98% of possible gammapathies because high resolution itself cannot, in some cases, discover overlapped monoclonal components for example of the Alfa2 or Beta zones. High resolution on Cellogel does not lose up to 50% of the monoclonal components present in patients as can occur with miniaturised micro electrophoresis and CZE.

It is true that the small monoclonal components are sometimes transitory but it is also true that in the case of amyloidosis AL, which can be confirmed by Bence-Jones IFE of urine, the patient presents only a small monoclonal component in the serum; it can also occur that patients who had a small monoclonal component at the moment of their six-month or annual control show multiplied immunoproliferation. Therefore, a high resolution system of electrophoresis, fast and reliable, such as that on Cellogel using manual or automatic methods, is needed.
MICROLONG HRE METHOD ON CELLOGEL, 5.7X14 cm

Microlong has already been mentioned on page 2 because of the critical reaction of the Finnish authors of the Aurora Hospital of Helsinki to the complicated “proposed selected method” of Jeppsson and Laurell. With a power supply, two tanks and an applicator, Microlong HRE enables from 8 to 48 tests to be performed in little over an hour (Fig. 11a).

Material necessary: Power Supply (code 10A08/B); 2 Tanks (code 11A11-CE); Micro applicator for 8 deposits (code 12A08/8P2); Rotating Shaker (code 13A34). It is advisable to have a Transilluminator (code 13A16).

Reagents necessary: Cellogel 190 microns, 5.7x14 cm (code 01A38-100); TGS Buffer (code 02A03-10); Coomassie BB 250 R stain; Destaining solution for Coomassie (475 ml methanol + 475 ml H₂O + 50 ml glacial acetic acid) and the drying whitener (code 07A02-S). There is a complete kit available for HRE code 08C31.

Method: 300 V migration for 35 minutes; staining time of 15 minutes; destaining time 1 bath of 5 minutes followed by 2 quick baths. The destaining is interrupted when the solution is still blue and substituted with a final bath of 5% acetic acid for conservation even in wet condition. The dry strip is obtained after immersion for 5 minutes in the whitening bath. The strip is placed on a glass plate and left to dry after removal of air bubbles and excess liquid with a glass rod. The strip can be treated with drying whitener and be observed and photographed with a digital camera on the transilluminator where the sensitivity limit of the Coomassie, with 90 ng/band per wet strip, is incremented up to a 20-30 ng/band (photomultiplication of the intensity of the microfractions). The report can be prepared utilising colour photocopies of the wet Cellogel placed between two transparent Mylar films or with a copy of the digital photo of the dried film placed on the transilluminator or with the image of the migrations scanned with Glob-Al Scan (fig. 11b). In this case the densitometric curve is not necessary. The molecular interpretation is not needed for the diagnosis of gammapathies. Nevertheless, it can be used, according to the indications of Laurell and of Aguzzi, when an erudite explanation is to be presented in medical circles, including family doctors particularly expert in dysproteinemia.
SEMI-MICRO HRE METHOD 18-36 TESTS ON CELLOGEL, 18.3X14 cm

Semi-micro HRE has the same sensitivity as the Microlong but is preferable for reporting results. With this method 18+18 HRE tests with semi-micro deposits of 0.7 μl, diluted 1+1 with the same buffer can be performed.

Material necessary: Power Supply (code 10A08/B); 2 Tanks (code 11A11-CE); semi-micro Applicator for 18 deposits (code 12A20/18P2); Rotating Shaker (code 13A34). It is advisable to have a Transilluminator.

Reagents necessary: Cellogel 190 microns, 18.3x14 cm (code 01E27-10); TGS Buffer (code 02A03-10), Coomassie BB 250 R stain, destaining solution for Coomassie (475 ml methanol + 475 ml H₂O + 50 ml glacial acetic acid) and the drying whitener (code 07A02-S).

Method: 300 V migration for 35 minutes; staining time of 15 minutes; destaining time 1 bath of 5 minutes followed by 2 quicker baths. The destaining is interrupted when the solution is still blue and substituted with a final bath of 5% acetic acid to conserve even in wet condition. The dry strip is obtained after immersion for 5 minutes in the whitening bath. The strip is placed on a glass plate and left to dry after removal of air bubbles and excess liquid with a glass rod. The strip can be treated with drying whitener and be observed and photographed with a digital camera on the transilluminator where the sensitivity limit of the Coomassie, with 90 ng/band per wet strip, is incremented up to a 20-30 ng/band (photomultiplication of the intensity of the microfractions). The report can be prepared utilising colour photocopies of the wet Cellogel placed between two transparent Mylar films or with a copy of the digital photo of the dried film placed on the transilluminator or with the image of the migrations scanned with Glob-Al Scan (fig. 11b). In this case the densitometric curve is not necessary.
The advantages of the Microlong and of HRE semi-micro in comparison with the high resolution method on French agarose: against a time of approx. one hour for 48 microlong tests or 36 semi-micro tests on Cellogel, the French method of HRE on agarose performs 10 tests per cycle and requires over 3 hours to do 36 tests with the defect of the floating and focused Beta-lipo which sometimes hides the monoclonals in the transferrin zone.

**Other Methods of High Resolution**

*Semi-automatic system on Celloclear Agarose Plus giving 54 tests/hour per sheet with the semi-micro technique.*

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**MICRO METHOD FOR UNCONCENTRATED URINE WITH SILVER BLUE STAIN (DISCOVERY OF BENCE-JONES AND CLASSIFICATION OF PROTEINURIAE)**

Electrophoresis of unconcentrated urinary proteins with Silver Blue Stain is altogether the most sensitive existing on the international market and it is our exclusive. The sensitivity of the method is double that of the Gold Stain methods and the affinity of the stains to the single proteins in the serum is sufficiently uniform as demonstrated by the comparative figure on page 33.

With simple micro applications of 0.3 μl/4 mm, without wasting time in costly operations of concentrating of the urine and using samples from extemporary urination, it is possible to discover myelomatose proteinuria, K free or Lambda free (Bence-Jones protein), thanks to the formidable colouring strength of the Silver Blue Stain. The latter is indelible in time contrary to what happens with the Silver Stain method on agarose. It must be underlined that the presence of the Bence-Jones protein in gammapathic patients serves as an index of malignity of the gammapathy. The patient is immediately directed to a Haematology and Transplant centre for the necessary treatment of the case. In the case of unconcentrated cerebrospinal fluid in micro scale the sample must be deposited 2 or 3 times on the start point.

**Materials necessary:** Power Supply (code 10A08/B); Tank (code 11A11-CE); micro Applicator for 8 deposits (code 12A08/8P2); Rotating Shaker (code 13A34).

**Reagents necessary:** Cellogel 190 microns, 5.7x14 cm (code 01A38-100); TGS Buffer (code 02A03-10); Silver Blue Stain **code 08C36/2**.

**Method:** 300 V migration for 25 minutes; the method for Silver Blue Stain is enclosed in the kit.
Simultaneous immunofixation of serum and urine of a patient who presents a monoclonal gammapathy for the first time with electrophoresis of the serum, should be the method chosen to classify and type the monoclonal component and to reveal the eventual presence of Bence-Jones in the urine (K free or Lambda free) with the use of K bound and Free antisera and anti Lambda Bound and Free without resorting to the use of uncertain and more costly anti-K free and anti-Lambda free. From the practical and organizational point of view of the laboratory with this method can be avoided the necessity to perform twice the IFE, of the serum and then of the urine with double expenses and double work time, as was imposed by the Beckman Paragon system. The method presented here respects the guide lines for IFE of the Bence-Jones proposed for urine alone with trivalent anti-serum (anti IgG + anti IgA + anti IgM), anti K Bound & Free and anti Lambda Bound & Free in the article published by Merlini et al. in Biochimica Clinica, 2001, Vol. 25, No. 1, pages 23-31, which we recommend be consulted.

Obviously, when electrophoresis of the urine of a gammapathic patient is performed, it is possible to obtain the patterns of myelomatose proteinuria (presence of one or more Bence-Jones bands), as well as the patterns of glomerular, tubular, mixed and post-renal proteinuria.

**Materials necessary:** Power Supply (code 10A08/B); Tank (code 11A11-CE); semi-micro Applicator 2+2 (code 12A02/SU); 5 Volumetric Distributors DC/3 (code 13A23); Rotating Shaker (code 13A34).

**Reagents necessary:** Cellogel 190 microns, 2.5x14 cm (code 01A08-100); TGS Buffer (code 02A03-10); Antisera anti-IgG, IgA, IgM, K and Lambda; Saline solution; Coomassie BB 250 R staining; destaining solution for Coomassie (475 ml Methanol + 475 ml H2O + 50 ml Glacial Acetic Acid); Clearing solution (code 06A06-S1). A complete kit code 08C09 is available.

**Method:** on the first strip buffered in TGS, marked SU, serum and concentrated urine (Minicon concentrator) are deposited simultaneously, while on the strips marked G, A, M, K and Lambda the serum diluted 1:5 and the concentrated urine are deposited. Electrophoresis at 300 V is performed for 30 minutes. At the end of migration the first strip is immersed in Coomassie staining; on the other 5 strips 40 µl antisera anti-IgG, IgA, IgM, K and Lambda are distributed using 5 distributors DC/3. After about 15 minutes incubation in the same migration chamber (emptied of the buffer solution) the 5 strips are washed in 2-3 baths of saline solution for at least 45 minutes to eliminate all the proteins which have not reacted. The immunofixed bands are revealed after staining with Coomassie and destaining solution. The wet strips are mounted on the serigraphed Mylar film included in the kit (code 08C09). See figure 3 on page 8.
IMMUNOFIXATION OF 6-8 PATIENTS ON 6 STRIPS OF CELLOGEL, 5.7X14 cm

With two tanks, a power supply and an applicator and kit code 08C09-2 complete with antisera, reagents and 5 Distributors DC/6, it is possible to perform the IFE of 6-8 patients in two hours.

Materials necessary: Power Supply (code 10A08/B); 2 Tank (code 11A11-CE); semi-micro Applicator 6 (code 12A08/6P2) or micro 8 deposits (12A08/8P2); 5 Volumetric Distributors DC/6 (code 13A24); Rotating Shaker (code 13A34).

Reagents necessary: Cellogel 200 microns, 5.7x14 cm (code 01A37-100); Tris-Hippurate buffer (code 02C13-2X-5); Antisera anti-IgG, IgA, IgM, K and Lambda; Saline solution; Amidoblack Staining (code 03C01-SC); Destaining solution for Amidoblack (475 ml methanol + 475 ml H₂O + 50 ml Glacial Acetic Acid); Clearing solution (code 06A06-S1). A complete kit code 08C09-2 is available.

Method: On the first strip buffered in Tris-Hippurate marked SP, the sera of the patients are deposited simultaneously, while on the strips marked G, A, M, K and Lambda the sera, diluted 1:5, are deposited. Electrophoresis is performed at 200 V for 30 minutes.

At the end of migration the first strip is immersed in the Amidoblack staining; on the other 5 strips, using 5 DC/6 Distributors, 100 μl of antisera anti-IgG, IgA, IgM, K and Lambda are distributed. The 5 strips, after incubation in the two migration chambers (emptied of buffer) are washed in 2-3 baths of saline solution for at least 45 minutes to eliminate all the proteins which have not reacted. The immunofixed bands are revealed after Amidoblack staining and destaining. The wet strips are mounted on serigraphed mylar film, included in the kit.

Figure No. 2 on page 7 illustrates the IFE of 6 patients. Execution time for IFE is about 2 hours, both for 6 and for 8 patients. There is no need for obsolete systems with absorbent sheets of filter paper, weights or presses.

METHODS FOR SIMULTANEOUS IFE FOR 1, 2, 4, 9, 18 PATIENTS: SEE CELLOCLEAR AGAROSE PLUS CATALOGUE

Universal Kit IFE for 1 patient on Celloclear Agarose Plus film using Beckman Paragon, Hydragel Sebia, Dako, Helena and Chemetron chambers.

A kit code 18A05-IFE 10.2x8.2 cm film and multiple applicator code 12-IFE1 are required.

The kit is predisposed for 10 tests on transparent films of Celloclear Agarose Plus. The removal of the proteins which have not reacted is effected with a simple wash in saline solution, as for Cellogel. The application of the serum and diluted serum is performed much more rapidly and safely than occurs with the frame systems, and the same can be said for incubation of the antisera which is realised overturning the film onto the capillary incubation plate code 12R03. Incubation time is only 10 minutes at room temperature, no incubation box is needed. The cost per test offers outstanding savings compared to what is offered by international competitors.
The method of electrophoresis of hemoglobin on Cellogel was drawn up by the Inst. De Biologie Clinique de l’Université de Paris by Drs. De Traverse, Coquelet and Percherp and is still today considered the standard reference method for the correct determination of quantitative anomalies of the hemoglobin (HbA₂, HbF). In particular, the quantitative determination of HbA₂ is practically impossible with micro electrophoresis due to the disproportion between HbA₂ (normal value ≤ 3.5%) and HbA (normal value = 96-99%). No densitometer can give a correct and repeatable value of HbA₂ in micro scale, and the problem is resolved adopting the semi-micro method where repeatability is satisfactory, and, in fact, the macro method used up to the 70s of electrophoresis with elution of the HbA₂ fractions in Drabkin solution followed by a spectrophotometric reading, has been abandoned. Semi-micro electrophoresis on Cellogel of the hemoglobins is a very simple technique which allows to reveal the qualitative (Hb S-C-D-E …) and quantitative (increase of HbF or HbA₂) anomalies. The separation of the hemoglobins is based on the properties of the electrical charge of the globin (heteroprotein associated with a prosthetic group called heme containing Fe). The globin can have a global positive or negative charge as a consequence of the different electrical charges of the amino acids which form the protein. Mutations of the globin chain modify the electrical charge and the different migration allows to recognize the anomalies.

**Materials necessary:** Power Supply (code 10A08/B); Tank (code 11A11-CE); semi-micro Applicator for 4 deposits (code 12A05); Rotating Shaker (code 13A34).

**Reagents necessary:** Cellogel 250µ, 5.7x14 cm (code 01A36-100); Tris Glycine buffer, dissolve in 1500 ml of H₂O, (code 02A01-10); Ponceau S staining; destaining solution Acetic Acid 5% or 3% Citric Acid. Clearing solution (code 06A06-S1). A complete kit code 08C35 is available. As this method on Cellogel, being comparative, does not require the use of abnormal hemoglobin as a standard. Only a normal fresh haemolysate is needed.
Preparation of the haemolysate: the blood, heparinized or treated with EDTA or other anticoagulants, must be used within a few hours of collection. The sample should be labelled with the identification data such as age, ethnic group, clinical and haematological observations, etc.
Centrifuge for 5 minutes at 3000 rpm. Remove the plasma. Wash the red blood cells suspending 1 volume of red globules in 4-5 volumes of NaCl 0.9% (saline solution), centrifuge and aspirate the supernatant. Repeat the operation 4-5 times. Lyse the red globules suspending 1 volume of red globules in 1.5 volumes H2O + 0.5 volumes toluene. Shake vigorously. Centrifuge again for 15-20 minutes and finally filter to obtain a perfectly limpid haemolysate. Photometrically control the titre of hemoglobin; it should be about 10 g per 100 ml. For semi-micro electrophoresis dilute 1 part haemolysate + 3 parts of H2O in order to obtain a concentration of 2.5%. With this dilution a precise densitometric quantitation of the HbA2 fraction can be achieved. Larger concentrations makes considerable disproportion between A2 and HbA and direct densitometry cannot be recommended.

Method: Immerse the strip in the buffer and agitate for 15 minutes. Eliminate any excess of buffer blotting the strips between 2 sheets of filter paper. Place the strips on the bridge and deposit the haemolysate at the negative pole. Perform electrophoresis at 200 V for 90 minutes. Stain the strips with Ponceau S for 5 minutes. Destain with 3-4 rinses in 5% acetic acid or 3% citric acid. In case of the quantitative determination of the A2 or HbF, proceed with rendering the strip transparent using the following procedure:
- 30 seconds bath of the Cellogel strip in the clearing solution (code 06A06-S1).
- Apply the strip to a Mylar film (code 13M01-100), with a glass rod remove excess solution and any air bubbles.
- Treat in oven at 80°C for 10 minutes.
Should the migrations present only qualitative anomalies, the so-called haemoglobinosis, it is sufficient to inspect the strip and the colour photograph of the same. The table below will help you to identify the position of abnormal hemoglobins in direct comparison with the pattern of normal hemoglobin.

<table>
<thead>
<tr>
<th>Condition</th>
<th>Allieic Character</th>
<th>Variant Hemoglobin</th>
<th>Variant HB</th>
<th>HbA2</th>
<th>Concentration HbF</th>
<th>HbA</th>
<th>Pattern</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Adult</td>
<td>-</td>
<td>-</td>
<td>3.5%</td>
<td>1.0%</td>
<td>96 - 99%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal Newborn</td>
<td>-</td>
<td>-</td>
<td></td>
<td></td>
<td>10 - 20%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sickle Cell Trait</td>
<td>Heterozygote</td>
<td>HbS</td>
<td>25 - 60%</td>
<td>normal or decreased</td>
<td>1%</td>
<td>25 - 60%</td>
<td></td>
</tr>
<tr>
<td>Sickle Cell Disease</td>
<td>Homozygote</td>
<td>HbS</td>
<td>80 - 100%</td>
<td>normal or decreased</td>
<td>4 - 12%</td>
<td>2 - 9%</td>
<td></td>
</tr>
<tr>
<td>Beta Thalassemia Minor</td>
<td>Heterozygote</td>
<td>—</td>
<td>—</td>
<td></td>
<td>4 - 12%</td>
<td>2 - 9%</td>
<td>slightly decreased</td>
</tr>
<tr>
<td>Beta Thalassemia Major</td>
<td>Homozygote</td>
<td>—</td>
<td>—</td>
<td></td>
<td>5 - 90%</td>
<td>20 - 80%</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin C Trait</td>
<td>Heterozygote</td>
<td>HbC</td>
<td>25 - 60%</td>
<td>negligible</td>
<td>—</td>
<td>25 - 60%</td>
<td></td>
</tr>
<tr>
<td>Hemoglobin C Disease</td>
<td>Heterozygote</td>
<td>HbC</td>
<td>80 - 100%</td>
<td>—</td>
<td>trace</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sickle Cell — C Disease</td>
<td>Heterozygote</td>
<td>HbC</td>
<td>20 - 80%</td>
<td>—</td>
<td>trace</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thalassemia — C Disease</td>
<td>Heterozygote</td>
<td>HbC</td>
<td>20 - 80%</td>
<td>4 - 12%</td>
<td>2 - 9%</td>
<td>20 - 80%</td>
<td></td>
</tr>
<tr>
<td>Thalassemia — Sickle Disease</td>
<td>Heterozygote</td>
<td>HbS</td>
<td>20 - 80%</td>
<td>5 - 8%</td>
<td>2 - 9%</td>
<td>20 - 80%</td>
<td></td>
</tr>
</tbody>
</table>
Interpretation

Normal pattern Adult subject:  
- HbA₂ ≤ 3.5%
- HbF ≤ 1%
- HbA = 96-99%

Normal pattern new born baby:  
- HbA₂ = 0%
- HbF from 90% to some marks at the age of 6 months.

Quantitative anomalies

1) Betatalassemia:  
b) Thalassaemia minor: partial synthesis of the Beta-chain. Presence of HbF. Increase of HbA₂ (4-12%).

2) Alpha-thalassaemia: absence of α chain.

Qualitative anomalies or haemoglobinosis

The haemoglobinoses are characterised by the presence of abnormal Hb bands (anomalies of the electric charge caused by the variations of the aminoacids).

1) Drepanocytosis or HbS: presence of a migrating band between the HbA and HbA₂ (alteration of the Beta-chain: AA Glu → val). Values are ranging from 80% to 100% for subjects with heterozygous state.

2) Haemoglobinosis C or HbC: presence of a very intense band at HbA₂ level (alteration AA Glu → lis). Variable values from 80-100% for subjects with homozygous state from 20% to 60% for subjects with heterozygous state.

A Hb fraction migrating to the level of the HbA₂ if superior to 12% does not indicate an increase of the A₂ but reveals the presence of abnormal C or E type haemoglobin. It is also possible to find together different types of anomalies like, for example, drepanothalassaemia or associated S and C haemoglobinoses. In these cases the diagnosis is established according to the percentages obtained from the densitometry reading.

HPLC is another qualitative-quantitative method for the determination of the hemoglobins. Unfortunately this first level method fails in some cases because of the co-elution of 2 or more genetic variants*. The use of the electrophoretic method to integrate the chromatographic method is indispensable. In all cases semi-micro electrophoresis on Cellogel as a first level analysis should be considered an optimal method together with other tests as, for example, the sickling test, isoelectrofocusing, the valuation of the haemochromocytometric parameters, etc.

ELECTROPHORESIS OF THE LIPOPROTEINS ON CELLOGEL, 5.7X14 cm

Use non-refrigerated fresh serum. Perform electrophoresis following the general instructions for the semi-micro (see chapter on how to use Cellogel, page 13) with 8.5 cm bridges and a semi-micro 4P applicator (code 12A05). Observe the serum, if it is evidently hyperlipemic deposit it only once, while, if it is limpid, deposit it twice on the start point. Use the usual Tris Hippurate buffer applying 200 V for 35 minutes.

At the end of migration immerse the strips in the Sudan Black B suspension (included in the Kit, code 08C42), prepared with 25 ml Solution A (staining) + 25 ml of Solution B (5% NaOH). Leave to soak for at least 45 minutes. The staining is complete after 2 hours. Wash the strip in running water for one minute. Place each strip between two Mylar films (code 13M01-100) and read by densitometer. Cellogel, during staining, becomes de-acetylated and is transformed into a film of hydrocellulose. Electrophoresis of the lipoproteins has lost importance because it has been substituted by automatic clinical chemistry tests of cholesterol, triglycerides, HDL, VLDL, LDL as well as some Apo-lipoproteins.

Electrophoresis of the lipoproteins however is today still a valid method when used by expert hands, for the evaluation of the atherogen and the classification of familial hyperlipoproteinemia according to Fredrickson. Cellogel rather than Agar or Agarose has made history in lipoprotein analysis with a large number of international publications. Literature and methods are available on request.

ELECTROPHORESIS OF GLYCOPROTEINS ON CELLOGEL, 5.7X14 cm

Perform electrophoresis following the general instructions for micro and semi-micro (see chapter how to use Cellogel on page 13) with 8.5 cm bridges and an 8P micro applicator (code 12A08/8P2) or a 4P semi-micro (code 12A08/4P4).

Use the Tris Hippurate buffer. Deposit the samples twice on the start point and make them migrate 20 minutes at 200 V for micro or 35 minutes for semi-micro.

When migration is concluded, fix in 95° ethanol for 5 minutes. Successively transfer the strips of Cellogel into a bath of 0.5% periodic acid (freshly prepared). Wash for 3 minutes in 3-4 baths of distilled water. Immerse for 15-20 minutes in Schiff reagent.
Preparation of Schiff Reagent:
Dissolve 2 g basic fuchsin in 400 ml boiling distilled water. Cool to 50°C and add 10 ml HCl 2N + 4 g potassium metabisulfite. Transfer the solution into a bottle of dark glass, cork it, leave for a night in the dark. Add 1 g activated charcoal destain and filter. Add 10 ml of HCl 2N, put one drop on a glass slide and leave to dry. Should the drop become red add a small quantity of HCl 2N to the solution and repeat the test on the slide until this dries without turning red. Keep the solution in the dark glass bottle corked and wrapped in black paper. Throw away if the solution becomes pink.
Wash in 6-7 rinses of 0.4% nitric acid (500 ml for 3-4 strips).
Fix in Formaldehyde at 40%. If the bottom turns pink the previous wash was insufficient, repeat the wash.
Final treatment of the strips: if the fractions are intense, proceed with clearing of the strip; if the fractions are weak, whiten the strip with whitening solution (code 07A02-S), dry on a glass slide, observe the whitened strip on the transilluminator and finally photograph it with a digital camera (Fig. 16).

SEARCH FOR ISOENZYMES
Cellogel, thanks to its characteristic of tolerating repeated deposits of serum sample and its characteristic which permits the treatment with liquid chromogen substrates without provoking chromatographic shifts of the separate fractions with electrophoresis, has outclassed all the dry cellulose acetates proving to be the best electrophoresis means for the study of isoenzymes both in the human field and in the animal and vegetable fields. In practice gelatinized Cellogel acetate has surpassed even agar and agarose and made the history of research of isoenzymes and allozymes, with the publication of books like “Allozyme Electrophoresis”, 1986, Academic Press, B.J. Richardson, P.R. Baverstock, M. Adams and “Enzyme Studies in the Interspecific Somatic Cell Hybrids” by Meera Khan, published by the Institute

**Electrophoresis of Lactate Dehydrogenase (LDH)**

Electrophoretic separation of LDH on Cellogel is very easy and can be performed by any laboratory technician. On the 5.7x14 cm strip, buffered in Tris Hippurate and placed on a France bridge (8.5 cm) two repeated semi-micro applications of unhaemolized fresh sera to be tested are made. In the meantime introduce into a thermostat at 37-40°C a dampened box with wet filter paper and a tight-fitting lid. The box should be able to contain at least one France bridge. A chromogen substrate is prepared dissolving in a test tube containing 1 ml distilled water the mixture of powders (Litium Lactate + NAD + MTT + Fenazine Metasulfate) of one capsule supplied into the LDH stain kit code 08C01. The powders must be perfectly dissolved; the test tube should be kept in the dark until use. The kit contains a DC/3 and a DC/6 volumetric distributor. After migration pipette 100 μl of chromogen substrate into the DC/6 distributor and apply it to the surface of the Cellogel still lying on the bridge passing the distributor up and down the zone occupied by the semi-micro migration. After 1 minute absorb excess substrate with squares of filter paper and then introduce the bridge with the strip into the incubation box inside the thermostat. Allow the reaction to develop for 30 minutes and then remove the box from the thermostat. If the isoenzymatic fractions appear too weak redistribute the chromogen substrate and as soon as the intensity of the bands is satisfactory transfer the film to a bath of 5% acetic acid. If 200 microns Cellogel is used, it is advisable to whiten the strip with the drying whitener (code 07A02-S) and observe it under the transilluminator. If 250 microns Cellogel is used, deposit the samples at start point 3 times instead of 2, the strip can be made transparent and then read on the densitometer.
**Interpretation:**

Normal Values:
- LDH1 22-36%
- LDH2 30-40%
- LDH3 16-2%
- LDH4 5-13%
- LDH5 4-8%

Pathological cases:
- LDH1 increase in acute myocardial infarction
- LDH2 increase in liver diseases
- LDH3 increases in pulmonary thrombosis, infectious mononucleosis and carcinomas
- LDH5 much increased with decrease of LDH 1,2,3: septicemia shock

Prof. Barnard at the Hospital of Johannesburg, on the occasion of the first heart transplant (13 December 1967), followed the patient with tests of the isoenzymes LDH in the serum on Cellogel thanks to his connection with the Presbyterian St. Luke’s Hospital of Chicago where Dr M. Kenny had perfected the first method of LDH on Cellogel.

**Electrophoresis of the Gamma GT**

Electrophoresis of the Gamma GT is a method used in centres for the control of alcoholics and laboratories connected to departments for the cure and treatment of patients suffering from hepatitis, cirrhosis and tumour of the liver.

The 5.7x14 cm strip of Cellogel, thickness 250 microns is buffered in Tris Tricine (code 02C02-6) for 10 minutes. On the Cellogel placed on the 8.5 cm bridge are deposited at 2 cm from the negative edge 4 samples of serum (fresh and unhaemolized) with a semi-micro applicator (code 12A05). The application should be repeated 4 times on the start point allowing the serum to be absorbed between each application. Effect migration at 200 V for 45 minutes. In the meantime introduce into a thermostat at 42°C a dampened box with wet filter paper and with a tightly closed lid. The box must be able to contain at least one France bridge.

Ten minutes before the end of electrophoresis, prepare the enzymatic substrate, dissolving the content of capsule A of the staining kit code 08C05 in a glass test tube containing 1 ml of distilled water. Heat the bottom of the test tube over the weak flame of a Bunsen burner, of a candle or a lighter. Shake while it heats until the powders are completely dissolved. At the end of electrophoresis remove the bridge from the chamber and distribute 150 μl enzymatic substrate on the Cellogel, moving the distributor up and down until the substrate is completely absorbed.

Place the bridge with the strip in the incubation box inside the thermostat, cover well and leave at 42°C for 40 minutes.

Shortly before extracting the box from the thermostat, prepare the staining solution dissolving the content of capsule B (Fast Blue) in 50 ml distilled water directly in a staining basin. Immerse the strip in the staining solution. The basin should be kept in the dark until the colour of the bands develops. Wash the strips 3 times in the destaining solution (acid acetic 5%). In the sera of alcoholic patients 4 fractions generally appear in the positions preAlb-Alb/Alb-α1/α2-α3/α2-β.
Percentage regarding the presence of fractions according to the diseases

<table>
<thead>
<tr>
<th></th>
<th>PreAlb - Alb</th>
<th>Alb-α₁</th>
<th>α₁-α₂</th>
<th>α₂-β</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Alcoholism</td>
<td>11.9</td>
<td>100</td>
<td>100</td>
<td>19.4</td>
</tr>
<tr>
<td>Toxicomania</td>
<td>15</td>
<td>97.5</td>
<td>97.5</td>
<td>17.5</td>
</tr>
<tr>
<td>Obstructive icterus</td>
<td>9</td>
<td>100</td>
<td>100</td>
<td>90.9</td>
</tr>
<tr>
<td>Hepatitis</td>
<td>0</td>
<td>100</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>16.6</td>
<td>100</td>
<td>100</td>
<td>13.3</td>
</tr>
</tbody>
</table>

Bibliography:

**Electrophoresis of Alkaline Phosphatase (AP)**

Electrophoresis of Alkaline Phosphatase is performed on Cellogel 5.7x14 cm, thickness 250 microns, buffered in Tris Hippurate for 15 minutes. Fresh sera, unhaemolized, are used and possibly a control serum which contains placental and hepatic enzymes. The best way to separate hepatic from osseous phosphatase is to incubate 100 μl serum plus 5 μl Neurominidase at 37°C for 30 minutes. In this case order staining kit code 08C02-N with Neurominidase.

Deposit the samples thus treated on the start point, at 1 cm from the negative edge, four times with semi-micro 4 deposit applicator (code 12A05).

Effect migration at 200 V for 40 minutes. Meanwhile place in a thermostat at 37°C a dampened box with wetened filter paper and tightly closed lid. The box must be able to contain at least one France bridge. A chromogen substrate is also prepared dissolving the content of a capsule A (naphthyl phosphate) in a test tube containing 1 ml of distilled water. Shake until completely dissolved and keep in a fridge until use.

After electrophoresis, remove the bridge from the chamber and distribute 150 μl enzymatic substrate on the Cellogel, moving the distributor up and down until the reagent is absorbed, eliminate the excess of liquid with little squares of filter paper and place the bridge in the incubation box inside the thermostat. Leave to incubate at 37°C for 30 minutes.

Shortly before extracting the box from the thermostat, prepare the staining solution dissolving the content of capsule B (Fast Blue) in 50 ml distilled water directly in a staining basin. Immerse the strip in the staining solution until the violet fractions appear (a few seconds). Transfer the strip into a bath of acetic acid at 5%. Photocopy or photograph the whitened strip placing it on the transilluminator with the normal procedures.
**Interpretation:**

Fraction 1: pos.\( \alpha_1 \) – isoenzymes of hepatic origin, greatly increased in hepatic diseases and is found in normal serum.

Fraction 2: pos.\( \alpha_2 \) – isoenzymes of osseous origin, increased in bone diseases and during period of growing in height.

Fraction 3: pos.\( \beta_1 \) – isoenzyme of placental origin, appears only during pregnancy.

Fraction 4: pos.\( \beta_2 \) – isoenzyme of hepatic origin, appears only in hepatic diseases when fraction 1 increases.

Fraction 5: pos.\( \gamma \) – isoenzyme of intestinal origin, it is the most cathodic fraction, appears only in diseases of intestinal origin.

A staining kit is available for Alkaline Phosphatase AP with a base of Bromo-Chloro-Indolyl Phosphate TNBT (Tetra Nitro Blue Tetrazolium).

There are about a hundred methods for Isoenzymes for which we can supply the bibliographical references of the Cellogel publications.

**HIGH RESOLUTION ELECTROPHORESIS ON CELLOGEL RS WEDGE OF UNCONCENTRATED URINE AND LIQUOR WITH NORMAL STAINS**

On page 32 of the catalogue Gold Stain and Silver Blue Stain systems to reveal the proteins of unconcentrated urine and liquor are described.

Historically, at the end of the 60s, electrophoresis of urine and cerebrospinal fluid and the proteic composition of normal and pathological samples was determined with the Cellogel “RS Wedge”, with deposits of the specimens in drop form (concentrated on the deposit point) of volumes of 25-50-100 \( \mu l \) and even 150 \( \mu l \). These volumes are in relation with the content of total proteins of the samples determined with litmus papers. (The use of the following table is recommended.)

<table>
<thead>
<tr>
<th>prot. concentration (g/L)</th>
<th>sample size (( \mu l ))</th>
<th>nanograms of proteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05 to 1</td>
<td>apply 150</td>
<td>7500 to 150000</td>
</tr>
<tr>
<td>1 to 3</td>
<td>apply 50</td>
<td>50000 to 150000</td>
</tr>
<tr>
<td>3 to 6</td>
<td>apply 25</td>
<td>75000 to 150000</td>
</tr>
</tbody>
</table>

Long and costly dialysis to concentrate the samples under examination from 20 to 150 times can be avoided.

**Materials necessary:** Power supply (code 10A08/B); Tank for Cellogel (code 11A11-CE); 3 bridges, 14 cm, (code 11B14); 25 \( \mu l \) pipette; Rotating Shaker (code 13A34); Transilluminator.
**Reagents necessary:** Cellogel RS Wedge 5x18.5 cm (code 01C11-25); Tris-Glycine in powder (code 02A01-10), dissolve one sachet in 1 liter of distilled water; Bromophenol Blue sample tracer stain (code 03A07-P), one crystalline to be dissolved in 1-2 ml of sample; Amidoback staining solution (code 03C01-SB); Destaining for Amidoback (475 ml H₂O + 475 ml Methanol + 50 ml Acetic Acid); Drying Whitener (code 07A02-S) to dry the destained Cellogel and to optically increase the sensitivity of invisible micro bands on wet or transparentized Cellogel.

**Method:** buffer one or three strips in Tris-Glycine for ten minutes while agitating. Remove the excess of the buffer between 2 sheets of filter paper, place the strips on the bridges with the penetrable surface facing upwards (the cut corner must be at the bottom on the right). The tail of the Cellogel must remain exposed by about 1 cm from the edge to allow the deposit even of a large drop of sample. Fix the ends of the strip with double paper wicks (4 small rectangles of filter paper 6x3 cm). Introduce the bridges into the chamber positioning the tail on the side of the negative compartment. Mark the strips 1-2-3 with a black biro. Deposit the first 25 μl of the three specimens on each strip, being careful that the strips are taut and horizontal, pipette further 25 μl doses according to the reference table. Cover the chamber and apply a voltage of 240 V for 2 hours 30 minutes. Observe the milliammeter, the initial current must be 2.5 mA per each strip. When migration is finished extract the bridges from the chamber avoiding dripping on the surface of the other strips. Immerse the strips in the bath of Amidoback staining for ten minutes, stirring, and destain in three baths until perfect destaining of the background is obtained. Transfer the strips into the whitening bath for 3 minutes. Gently place the strips on a well-cleaned glass plate or on a Mylar film 18.3x14 cm (code 13M04-100) and leave to dry at room temperature. When the strip is perfectly dry it can be observed on the transilluminator. After optical enhancement, photograph the results in a dark room.
Interpretation of the results: compare the result with the typical images.

2. Myelomatous Proteinuria: presence of Gamma fractions, also monoclonal, and absence of other proteins or weak evidence of Albumin and other fractions.
3. Bence-Jones Proteinuria: similar to the previous one with weak fractions (K free and Lambda free), confirmable with IFE.
5. Non-selective glomerular Proteinuria: as for previous one, with presence of \( \alpha_2 \)-macro between Transferrin and Albumin.
6. Tubular Proteinuria: presence of tubular components such as microglobulins in Gamma zone.
7. Mixed Proteinuria: Glomerular + Tubular components.
8. Post-renal Proteinuria (blood in the urine): pattern similar to that which would be obtained with serum.

The study of urinary proteins with Cellogel RS was the object of articles, decisive for knowledge of renal damage and today, too, these studies render it possible to avoid kidney biopsies or tests that are more complex and costly than simple electrophoresis of unconcentrated urine. The most important authors of articles on urinary proteins have been such famous nephrologists as Prof. Maiorca, Prof. Scarpioni, Dr. Alessandrino, Prof. Brancaccio and Dr. Heer in Argentina.

**Electrophoresis of the Cerebrospinal Fluid** is analogous to that of unconcentrated urines, normally in this case the deposit is not less than 100 \( \mu l \). The pattern of normal liquor is similar to that of normal serum but with a strong pre-Albumin band and weak presence of gammaglobulins and slight \( \beta_2 \) band and transferrin typical of the liquor (carbohydrate deficient). In the case of multiple sclerosis and diseases of the central nervous system (CNS diseases) the migration shows numerous band of IgG oligoclonals, immunofixable with Anti-IgG, in this way the revelation sensitivity of
the oligoclonal band is amplified about 5 times. Immunofixation is effected cutting a long strip of Cellogel RS in half lengthwise. While one half is being stained with the stains used as for urine, the other half is immunofixated with 50 μl Anti IgG distributed with a DC/3 distributor (code 13A23) in the gamma zone. After 15 minutes incubation, the strip is washed three times in saline solution for not less than 45 minutes and then stained with Amidoblack or Coomassie. The following figure shows a case of immunofixation of transferrin obtained using 50 μl of anti-Transferrin antiserum.

Electrophoresis and immunofixation of CSF on half strip. 4 to 5 bands of Transferrin are shown with anti-Transferrin antiserum.


Note: Those laboratories that do not have the problem of the cost of Minicon concentrators and their relative use can utilise the semi-micro HRE electrophoresis method described for serum proteins on page 37 of the catalogue depositing samples of concentrated urine or liquor.
12. THE USE OF CELLOGEL RS IN THE ANALYSIS OF ALIMENTARY PROTEINS

ELECTROPHORESIS OF PROTEINS OF FLOUR OF COMMON WHEAT, DURUM WHEAT AND MIXTURES

With this method it is possible to determine the presence of common wheat in percentages lower than 5% in mixtures with durum wheat and also in pasta. The proteins are extracted using a Tris Glycine buffer. Place 1 g of flour in a test tube and add 2 ml Tris Glycine. Stir with a glass rod, pressing the sides of the tube. Leave for one night. Centrifuge for a few minutes until a limpid supernatant is obtained which is suitable for electrophoresis.

To perform electrophoresis on Cellogel RS Wedge, 5x18.5 cm, a Cellogel tank is needed (code 11A11-CE) with 3 bridges, 14 cm (code 11B14). The same protocol as indicated on page 49 for electrophoresis of unconcentrated urine is used. Pipette 6 μl of the protein extract onto the deposit point. Effect migration at 300 V (2.5 mA per strip) for a time of 90 minutes. Stain in Amidoblack for 10 minutes and destain in three washes of methanol, distilled water, acetic acid (475:475:50 ml); if necessary wash again to obtain a perfectly white background. Whiten and dry the strip following the procedure relative to the use of the whitener (code 07A02-S). When the strip is perfectly dry it can be placed on the transilluminator to observe the photomultiplication of the intensity of the microfractions, previously invisible, and the pattern obtained can be photographed.

HAEMOLYMPH OF FLOUR WORMS

Tris Glycine buffer. 14 cm bridge. 2 applications of 1.5 μl of the haemolymph drawn from 2-3 worms. The worm must be at that stage of life when it can move easily. The worm is pricked with a needle and the micro-drop of haemolymph is aspirated with a microcapillary. Migration of 300 V for 90 minutes.

SERUM OF MILK

TGS buffer (Tris Glycine Salicylic Acid). 14 cm bridge. 3-4 applications of 1.5 μl. Migration at 360 V for 80 minutes. It is better not to add precipitant reagents, but ultrafiltrate the milk on a membrane under aspiration (the membrane is made of a sheet of Cellogel and retains the macromolecular of casein while allowing the proteins of the milk serum to filter through). The limpid filtrate
obtained for electrophoresis is casein-free. On Cellogel RS the Serum albumin M.W. 69000 runs in first position while on Polyacrylamide stays in third position held back by the molecular filtering action.

**MILK CASEIN**

Buffer for the impregnation of the strips and to dissolve the casein: 76.9 ml NaVeronal 0.1 M + 23.1 ml HCl 0.1 N + 200 ml H₂O (pH 8.2 – 0.033 M) + 90 g Urea.

Buffer in the chamber: NaVeronal 8.24 g + 75 ml HCl 0.1 N per 1 liter.

2 applications of 1.5 μl of specimen at 15%. 14 cm bridge. Migration at 300 V for 90 minutes.

**PROTEINS OF SEEDS OF PEANUTS, OF SOYA, ETC.**

Use the same technique as for proteins of flour.

**PROTEINS OF ANIMAL TISSUES (MEAT AND FISH)**

1 g of tissue is homogenated with 2 ml Tris Glycine buffer. Centrifuge at 5000 rpm until a limpid supernatant is obtained even if coloured, this contains the soluble proteins of animal tissue. Rectangular 5.7x18.5 cm Cellogel RS (code 01C04-100) and a semi-micro applicator (code 12A05) are used. Tris Glycine buffer (code 02A01-10). 14 cm Bridge. 3-4 applications of 1.2 μl of sample. Migration at 300 V for 90 minutes.

**REVELATION OF THE ESTERASES**

Perform electrophoresis as described for animal tissues and five minutes before the migration is concluded, prepare the following solution: 120 mg alpha-naphthylacetate (Sigma) + 5 ml acetone + 15 ml ethanol. Completely dissolve and add 10 ml distilled water. Immerse the strip for an instant in this solution and spread it on a glass plate. Put the plate in the tank, placing it on the bridge. Cover and leave to incubate for 30 minutes. One minute before the incubation time expires, dissolve 30 mg Fast Blue R.R. in 30 ml of distilled water. Immerse the strip for an instant in this solution and transfer it immediately to a water rinse. The enzymatic fractions de-acetilate the alpha-naphthylacetate during incubation and the de-acetylate reacts with O-dianisidine tetrazotized giving an insoluble violet compound. The search for the esterases is undertaken on the total extracts. With this system it is possible to type and recognize the genetic variants of individuals of the same species. This research is of great importance for distinguishing different types of meat. Methods and reprints of the national and international scientific publications on electrophoresis of alimentary proteins mentioned above are available on request.
13. ISOELECTROFOCUSING ON METHYLATED CELLOGEL

AMBLER TECHNIQUE (UNIVERSITY HOSPITAL QUEEN’S MEDICAL CENTRE, NOTTINGHAM, ENGLAND) AND C. RINALDI, G. DE GENNARO AND G. ZANNI (LABORATORIO PATOLOGIA CLINICA, TEANO, 1997)

Methylated Cellogel, according to Ambler, after impregnation in mixtures of ampholytes (e.g. mix pH 3 – pH 9) lends itself to the separation of the proteins according to their isoelectric point. To perform the process a commercial refrigerated tank for Isoelectric focusing (Isolab-USA and others) is required, together with a power supply at high voltage (at least 1000 V). At this voltage the methylated Cellogel gives a null electroendosmosis. The detailed method is supplied for the methylation of at least 10 strips of Cellogel, 5.7x14 cm, or sheets of other sizes for every operation. The method for the impregnation of the film in ampholytes with a different range of pH to perform the separations of the variants of the α1 antitrypsin of the immunoglobulins, of the liquor, of the hemoglobins, etc. of the various European producers is also included.

The haemoglobin method drawn up by Dr Rinaldi and his collaborators is particularly interesting for the study of the haemoglobinopathies of patients immigrated from South-East Asia where instead of HbA2 performed with traditional electrophoresis HbE and other qualitative anomalies are found.
14. IMMUNOTECHNIQUES ON CELLOGEL

IMMUNOFIXATION OF MONOCLONAL COMPONENTS


IFE methods are described on pages 39-40.

IMMUNOFIXATION OF CRYOGLOBULINS

Cryoglobulins are identifiable in the samples of sera subjected to electrophoresis at high resolution on Cellogel because these leave a deposit mark at the start point (and this does not occur on agarose with filtrating applicators, a serious defect of the French system). Proceed to IFE of the cryoglobulins after having washed and dissolved the cryoprecipitate in a buffer containing urea.

Three types of Cryoglobulins exist:
Type I is a monoclonal immunoglobulin which is present in multiple myeloma or in Waldenström’s macroglobulinemia and can be identified with IFE.
Type II is an IgM monoclonal aggregate with IgG policocolonal which can be identified with IFE.
Type III is an aggregate of IgG, IgA, IgM policlonals and represents 50% of the cryoglobulinemiae. The cryoprecipitate which can be obtained after a week of treatment at 1-4°C is modest. IFE does not reveal the monoclonal band. The cryoglobulins of Type III, when the patient is affected by chronic infections (e.g. Hepatitis C), have a cryoprecipitate which can be mixed with immunocomplexes (e.g. of IgM policlonal with virus and bacteria).

IMMUNOFIXATION OF THE C₃ ACTIVATION PRODUCTS

IMMUNOFIXATION OF OTHER PROTEINS OF THE SERUM

In many cases IFE has proved itself useful after high resolution electrophoresis to exclude the eventual presence of a monoclonal component, for example in position β₁. Working in high resolution it is possible to see two variants of Transferrin split in position β₂. To eliminate any doubts it is possible to immunofixate the Transferrin with anti-Transferrin as done with the liquor (see page 51). If the anti-Transferrin immunofixates on the double band it is evident that there are two variants of the Transferrin. If it immunofixates on only one band, the second band could be a monoclonal component which can be confirmed with normal IFE of the immunoglobins. IFE has also been applied to immunofixate the variants of Haptoglobin, when searching for the GC protein and other single proteins.

IMMUNOFIXATION OF THE MINOR COMPONENTS OF THE SERUM

The future of electrophoresis is the research with high resolution and immunofixation of the minor protein components of the serum. In fact, 1% of the serum proteins is made up of dozens of proteins, subordinate to the 5 zones of normal electrophoresis, known as minor components. These also include tumour markers like PSA and CEA or alpha-fetoprotein and non-tumoral markers expressed by non-proliferating cells in certain pathological states or genetically anomalous of the patients. The development of immunoelectrophoretic test for immuno-visualization of groups of several minor components for varying diagnostic scopes is foreseeable.

MOST-ICA MEMBRANE ORDINATE SPOT TEST – IMMUNOCHEMICAL ASSAY

This method exploits the fact that Cellogel is a filtrating membrane to perform immunofixation on spots of 5 μl applied tidily (48 spots on a Cellogel membrane, 5.7x7 cm). One protein at a time is determined quantitatively in concentration such that 5 μl serum, diluted and deposited, contain about 100 ng of the protein being searched for 6 of the 48 positions of the spots are dedicated to the calibration scale which indicates 0-50-100-150-200-250 ng. Up to 42 samples can be analysed immunofixating in polyclonal antiserum prediluted 1:10 – 1:20 depending on what antigen is to be determined. After 20 minutes incubation the film is washed in saline solution (about 100 ml) on the Filtrawasher (code 13A44) for 2-3 minutes. If no Filtrawasher is available, wash for about 1 hour in three washes of saline solution. Stain with Amidoblack, destain and clear, read on Glob-Al Scan which offers 8 sequences of peaks corresponding to the spots of the calibrators and to the spots of the samples. As the reading is in optical density the height of the peaks is directly proportionate to the concentration.

The MOST-ICA method is a low cost alternative to immuno-turbidimetric and immuno-nephelometric methods of determining single proteins.
ELECTROIMMUNODIFFUSION OR ROCKETS OR LAURELL’S TECHNIQUE FOR THE QUANTITATIVE DETERMINATION OF SINGLE PROTEINS

Electroimmunodiffusion is a technique of immunoprecipitation in an electric field on Cellogel or agarose. The method is intended for the quantitative determination of single proteins which react with their specific anti-serum (for example, albumin of serum against specific anti-albumin). Samples and calibrators are applied, in the most opportune concentration, on the Cellogel strip impregnated with the specific antiserum (the samples can be deposited prior to the distribution of the anti-serum). Applying a low voltage of 120 V, the antigen migrates electrophoretically into the field of the antibody producing a peak of immunoprecipitate (rocket) the height of which is proportional to the concentration of the antigen. The EID technique is used to control the kits of immuno-nephelometry and of immuno-turbidimetry and for the determination of antigens for which no commercial kits exist.

Materials necessary: Power supply (code 10A08/B); Tank (code 11A11-CE); 1 μl Microcaps complete with Tygon tube; Plastic Guide for Cross-over and Laurell Technique (code 13A29); Volumetric Distributor DC/6 (code 13A24); Rotating Shaker (code 13A34); Plastic Box for washes (code 13A50).

Reagents necessary: Cellogel 5.7x14 cm, 200 microns (code 01A37-100); Tris Glycine buffer (code 02A01-10), dissolve the contents of the packet in 1000 ml distilled water; Antisera anti-human proteins; Bromophenol Blue (code 03A07-P); Amidoblack (code 03C01-SC); Destaining solution (475 ml Methanol + 475 ml distilled water + 50 ml Acetic Acid); Clearing solution (code 06A06-S1).

Method: Immerse the strips of Cellogel in the buffer for 15 minutes. Load the volumetric distributor DC/6 with the opportunely diluted antiserum (see table).

The following table shows the quantities of rabbit antisera and related sample and standard dilutions that are necessary for one Cellogel strip 5.7x14 cm

<table>
<thead>
<tr>
<th>antiserum</th>
<th>μl</th>
<th>μl/cm²</th>
<th>standard dilutions</th>
<th>samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>anti pre-albumin</td>
<td>50</td>
<td>1</td>
<td>1/2 1/4 1/8</td>
<td>1/4</td>
</tr>
<tr>
<td>anti albumin</td>
<td>100</td>
<td>2</td>
<td>1/20 1/40 1/80</td>
<td>1/40</td>
</tr>
<tr>
<td>α1 antitripsin</td>
<td>100</td>
<td>1</td>
<td>1/2 1/4 1/8</td>
<td>1/4</td>
</tr>
<tr>
<td>anti orosomucoid</td>
<td>50</td>
<td>1</td>
<td>1/2 1/4 1/8</td>
<td>1/4</td>
</tr>
<tr>
<td>anti Lp(a)</td>
<td>50</td>
<td>1</td>
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<td>1/4</td>
</tr>
<tr>
<td>anti α2 macroglobulin</td>
<td>50</td>
<td>1</td>
<td>1/2 1/4 1/8</td>
<td>1/4</td>
</tr>
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Dilute the standards and the specimens with saline solution stained with Bromophenol Blue. Place the strip between two sheets of filter paper to remove the excess buffer. Position the strip on the bridge with the penetrable surface facing upwards. Before transferring the bridge into the tank, position the plastic guide for Cross-over on the bridge and deposit the specimens using the 1 µl Microcaps. Act rapidly to avoid dehydration of the strip. Apply the specific antiserum on the whole surface passing the volumetric distributor up and down until the liquid is completely absorbed. Homogeneous distribution is obtained by loading the volumetric distributor with 150 µl of total volume (e.g. 100 µl of Tris Glycine + 50 µl of anti pre-albumin). Position the bridge in the chamber with the start point in the zone of the negative pole, with the exception of the IgG which is placed in the zone of the positive pole so that it retromigrates towards the negative pole (thus forming a peak of immunoprecipitate). The use of the Microcaps requires a little prior practice. Given the importance of the Microcaps for the satisfactory outcome of the analysis, practice before their utilisation is the object of the training courses held at our Milan laboratories.

Apply 120 V. Migration time: preferably a whole night (Once the peak of immunoprecipitate is formed, it remains blocked even if the power supply remains connected for several hours longer than needed). For albumin and proteins having mobility of the α1, the migration time can be reduced to 5 hours. After migration, wash the strips in 4 baths, ten minutes each, of saline solution to remove the unreacted proteins (excess of antiserum and various proteins of the sample). Immerse the strips in Amidoblack for ten minutes, and destain. Keep the strips in a bath of 5% acetic acid. Clear or dry after having measured the height of the peaks. Alternatively it is possible to effect rapid staining without a decolorizing bath: after migration and the wash in saline solution, immerse the strips in the following solution with low concentration of green stain: 0.03 g green Lissamine in 45 ml methanol + 45 ml H2O + 10 ml acetic acid. Conserve the strips in a bath of 5% acetic acid.

**Results:** on graph paper draw the calibration curve with 3 standards. The calibration curve can be either a straight line or a concave line. Interpolate the heights of the samples to obtain the concentration.

**Possible errors:** too high and open a peak means there is an excess of antigens. Too low a peak means an excess of antiserum or too low concentration of antigen. If the peak is barely visible, this is due to scarce affinity or avidity of the antiserum.
CROSSOVER ELECTROPHORESIS

Crossover for research into the infectiousness of antigens for which no commercial antisera exist; e.g. Antigen Australia later classified as Hepatitis B

6 aligned drops of 5 µl of sera of the patients to be tested are deposited in the centre of the strip with the aid of the guide. 1 cm apart, in the positive zone, 6 drops of 5 µl of the antiserum against the antigen being looked for. At the negative electrode, 1 cm apart, 6 drops of 1 serum definitely containing the antigen is deposited. This can react with the antibodies of the patients thus making it possible to find the antibodies while an eventual antigen will immunoprecipitate from the retromigrating immunoglobulins of the antiserum. This technique was widely used for the Australia antigen, before this was indentified as Hepatitis B virus and is still used to examine sera of patients suspected of containing infectious agents even when no commercial antigen antibodies exist. In this case use the sera of infected donors. The technique is also suitable for detecting tumoural markers (for example the alpha fetoprotein for which there are specific polyclonal antisera). The Crossover is performed at 200 V for about 20 minutes or even less and is at least 4 times more sensitive than the Ouchterlony radial diffusion technique. All the antigens of the specimen and all the immunoglobulin of the antiserum are conveyed so that they collide and react forming a little line of immunoprecipitate after a few minutes of electrophoresis. Successively the strip is washed in saline solution to eliminate the proteins which have not reacted and stained in Amidoblack or Coomassie to reveal with high sensitivity the immunoprecipitate which has formed in the collision between the retromigrant antibody and the migrant antigen towards the positive pole.

Crossover for ENA Autoantibodies

It is important to illustrate an ENA autoimmunity test. The same reagents are used as those contained in the kits supplied by producers of the IFA test for autoimmunity. These kits are intended for performing the ENA test with the Ouchterlony method on a plate of agarose in 18-24 hours and offer limited sensitivity. Using the same reagents on buffered Cellogel and with the aid of the Crossover guide the ENA test is completed in about 90 minutes (15 minutes Crossover at 300 V + 45 minutes washing in saline solution + 10 minutes staining + 5 minutes destaining and colour photography of film). A rapid result is obtained within the day and the number of tests that can be performed with the reagents supplied in the IFA kits is doubled or trebled.

Crossover for the screening of infected populations

Crossover on sheets of Cellogel 18.3x17 cm (code 01E33-10) lends itself to screening of infected populations in case of epidemics. A memorable example is the meningitis epidemic which occurred in St. Paolo of Brazil in 1973. The Adolfo Lutz Institute knew about the Crossover method on Cellogel because they applied this technique for the identification of the Australia antigen (Hepatitis B). When the very serious meningitis epidemic occurred involving children of school-age in the Brazilian megalopolis, the doctors of the Lutz Institute, after having consulted Dr Del Campo in Milan, within a few days had tested thousands and thousands of children using
Cellogel and analysing 48 samples per sheet. The samples of the children's cerebrospinal liquid were applied in 4 rows and the same number of opposite deposits were made with anti Neisseria Meningitidis group C antiserum in 4 rows 1 centimeter apart. Crossover Electrophoresis at 200 V for 4 minutes. Rinse in saline solution for 1 hour. Stain in Amidoblack and destain.

**Crossover with High Sensitivity**

To increase the sensitivity of the Crossover to levels comparable with those of the immunoenzymatic, after normal Crossover in duplicate, one with IgM and the other with IgG between patient's serum and antigen, after washing in saline solution, the 2 Cellogel strips are buffered in PBS (pH 7.2) for ten minutes, blotted to remove excess buffer and placed on the bridges. On the first strip 50 μl fluorescein antiserum anti-human IgG, diluted according to the producer's recommendations, are deposited in the reaction zone (in the centre). On the second strip 50 μl fluorescein antiserum anti-human IgM, diluted according to the producer's recommendations, are deposited in the reaction zone (in the centre).

After 15 minutes of incubation the two strips are washed in saline solution (3 baths of 10 minutes under agitation) to eliminate the background fluorescence. The strips are observed with a UV lamp at 254 nm. The presence of a line of an immunoprecipitated IgG signifies a recent infection while the presence of a line of an IgM signifies past infection. This test, used for example for the toxoplasmosis test in pregnant women, has never missed a single real case of IgM positivity compared with other methods. It is possible to obtain good staining results using, instead of fluorescein antisera, peroxidase stained rabbit antibodies anti-human IgG and anti-human IgM. In this case the lines of immunoprecipitate are coloured brick red with the solid chromogen AEC.

**OUCHTERLONY TECHNIQUE:**

**Qualitative Radial Immunodiffusion**

The Ouchterlony technique was born on agar contained in Petri dishes with a diameter of 6-7 cm. Generally the agar was perforated with a well in the centre and 5 wells positioned on the circumference, about 1.5 cm from the centre. This is a qualitative radial immunodiffusion method that lasts 18-24 hours. The antiserum is applied in the centre while the sera of the patients, in which the detectable antigen is to be identified, are pipetted in the 6 peripheral wells. The serum of the patient can be deposited in the centre and then look for 6 different antigens by pipetting 6 different antisera in the peripheral wells.
This technique was adopted in the 60s using Cellogel buffered in PBS (pH 7.2) stretched between two clamps in a perfectly horizontal position, all contained in a damp box. Successively Cellogel was substituted by Cellogel/Myl, 6x7.6 cm (Cellogel supported on a Mylar film). The buffered film is placed on a sheet of graph paper on which the central and peripheral deposit points have been drawn. It is sufficient to place the buffered film on the drawing to be able to see the deposit points in semitransparency. 4-5 µl sera or antisera are used. The film is left to incubate for 18-24 hours inside a small box (code 13A51), the bottom of the box is dampened with a sheet of wet filter paper while the Cellogel/Myl film is held raised at the extremities with two pieces of polystyrene 10 mm thick. Cover and leave to incubate at room temperature. Successively the film is washed in saline solution for 4 baths of ten minutes. Stain with Ponceau S or Amidoblack and destain.

**MANCINI TECHNIQUE:**
Quantitative Radial Immunodiffusion

The technique of Mancini and Carbonara was invented at the University of Turin and proposed on agarose fused with specific antisera in 10 cm petri dishes predisposed with perforated wells. The system commercialised by various companies was, and still is, costly because the plate requires at least 4 µl per cm² antiserum, furthermore, the product expires within a few months. The major drawback of the system, however, lies in the fact that a laboratory which uses these plates creates voluminous piles of used Petri dishes which cannot be thrown away until they have been examined with an ocular micrometer with which the diameter of the circle of the immunoprecipitate is determined and is confronted with the concentration of the determined protein. The method was transferred onto Cellogel by Profs Vergani and Agostoni of the Policlinico in Milan and by Spanish researchers. It was adopted by some suppliers who created a business which is still going strong in some countries with a tropical climate. As in the case of the Ouchterlony technique, Cellogel was substituted by Cellogel/Myl (supported by Mylar) for its functional capacity. The preferred film is therefore Cellogel/Myl, 6x7.6 cm, on which 10 tests, including 4 calibration points, can be performed.

Cellogel/Myl, respect to agarose, requires a quarter of the antiserum needed to obtain quantitative immunoprecipitate circles. All the proteins of the serum can be determined with immunoturbidimetry using only 1 µl per cm² instead of 4 µl per cm² needed for agarose. Furthermore, the Cellogel Immunofilm is freshly prepared each time. The preparation is very simple, all that is needed is a DC/6 Distributor (code 13A24) and the appropriate antisera. Cellogel is washed in PBS for ten minutes, the excess buffer solution is removed with filter paper, then 45 µl of antiserum (preferably stained with a Bromophenol Blue crystalline) are distributed on it and it is placed horizontally in the small damp box (code 13A51). At the moment of use the film is placed on the drawing through which the deposit points can be seen, then 1 µl deposits of the opportune diluted protein are made, using the Microcap mounted on the Microcap holder furnished with a little Tygon tube on which one can blow delicately to inject the sample onto the deposit point. Consult the Laurell technique and relative table for the dilution of the samples and the calibrator. Cover the incubation box, allow to diffuse for 24 hours at room temperature. Wash 3 times in saline solution. Stain with Amidoblack and destain. At this point place the film on the transilluminator and measure the diameter of the circles with the ocular micrometer. Construct the calibration curve with the 4 calibrators and then draw up the report on each patient. With this method there is an
enormous cost saving per test and at the same time conservation problems of the traditional immuno-plates are avoided as well as their elimination. Cellogel/Myl films, duly numbered and dated, can be stored without creating problems of space.

TWO-DIMENSIONAL IMMUNOELECTROPHORESIS

Two-dimensional immunoelectrophoresis was proposed by Scandinavian researchers on agarose. The method was very complicated because two pourings of agarose were needed, one without antisera for the migration of the serum protein in the first dimension, and the second pouring containing total antisera for the second dimension. The operation required carbamilation of serum samples to avoid the retromigration of IgG, IgA, IgM. A refrigerated chamber with circulating water was necessary as well as very long staining and destaining times of the agarose, 1 mm thick.

The sheet of Cellogel immediately proved ideal for the two-dimensional immunoelectrophoresis technique. A volume of antisera equal to a quarter of that needed on agarose is required. The method became 4 times more sensitive to that on agarose, carbamilation (toxic) was not necessary because, on Cellogel with a deposit of the serum next to the negative edge, the immunoglobulins migrated towards the positive pole without retromigration. This was also due to the chromatographic action of the thrust towards the centre of all the proteins. Cooling of the chamber with the circulation of water was not required because the thin film of Cellogel of 200 microns dissipated the heat generated by the Joule effect at room temperature, despite the fact that the time required for the second dimension being at least 5-18 hours. Thanks to these facilitations two-dimensional immunoelectrophoresis made a great contribution to research and knowledge of serum proteins and spread in the Scandinavian countries, above all in the laboratories of control of the antisera production. Two-dimensional immunoelectrophoresis has not had much success in hospitals because the method is not applicable to large numbers of samples. Two-dimensional immunoelectrophoresis remains valid for liquor and urine.
**Materials necessary:** Power supply capable of erogating 200 V for the first dimension and 120 V for the second dimension (code 10A08/B); Tank (code 11A11-CE) with bridge (code 11B03); Plastic guide for two-dimensional electrophoresis (code 13A26); Volumetric Distributor DC/6 (code 13A24); Drummond Microcaps of 0.5 µl and of 1 µl with Tygon tube; Rotating Shaker (code 13A34); Plastic Box for staining and destaining.

**Reagents necessary:** 14x14 cm Cellogel sheets (code 01E06-10); Tris Glycine buffer (code 02A01-10); Coomassie BB 250 R stain; Destaining solution (475 ml distilled water + 475 ml methanol + 50 ml acetic acid); Bromophenol Blue in powder to stain the specimen (code 03A07-P); Saline solution (NaCl 0.9% in distilled water); Destaining solution 5% acetic acid; Sheets of Mylar (code 13M04-100); Clearing solution (code 06A06-S1); Rabbit antiserum Anti-Human total SP pre-stained 1 ml (code 19S01-2).

**Method:** Equilibrate the sheet of Cellogel in Tris Glycine buffer for 15 minutes (dissolve 1 sachet in 1 litre of distilled water). Fill one compartment of the tank with Tris Glycine buffer and level. Dilute the sample of the patient’s serum 1:4 with saline solution coloured with Bromophenol Blue. Remove the excess of buffer from the Cellogel using 2 sheets of filter paper. Position the sheet of Cellogel on the 8.5 cm bridge with the penetrable surface facing upwards (recognizable by the embossed Cellogel inscription), apply the plastic clips. Do not introduce the bridge into the tank before having applied the specimen. With the 0.5 µl Microcaps apply the specimen of diluted serum (helping yourself with the plastic guide) in the cathodic zone at 8 mm from the negative edge and at 35 mm from the end of the sheet. Practice depositing with Microcaps using a small strip of buffered Cellogel and the Microcaps filled with blue-stained protein solution blowing delicately into the Tygon tube. Introduce the bridge into the tank taking care to position it with the deposit on the negative pole, cover the tank. Apply 200 V for 55 minutes. Application of the antiserum and second migration.

Remove the bridge with the sheet of Cellogel from the chamber. Dispense 100 µl total antiserum in the DC/6 distributor, add 50 µl saline solution to obtain a volume of 150 µl. Use the guide to distribute the antiserum on the sheet passing up and down with the DC/6 distributor until the antiserum is completely absorbed. Dismount the sheet from the bridge, turn it round 90°, and then remount it so that the trace left by the bromophenol during the first
migration is 5 mm from the edge of the bridge. Introduce the bridge into the tank with the traces of bromophenol at the negative pole. Apply 120 V for 16 hours (1 hour more or less does not influence the final result). Wash the sheet in saline solution, 6 baths of 15 minutes while agitating. Stain with Coomassie for 30 minutes. Destain for 3-4 baths of 5 minutes. Stop the destaining of the methanol solution by transferring the sheet into a bath of 5% acetic acid. The sheet can be preserved in this solution for 2-3 days. Place the sheet between two films of Mylar, dry the excess liquid and photograph or colour photocopy.

Treat the sheet in a clearing bath (code 06A06-S1) for 3 minutes. Stretch the sheet on Mylar film, eliminate the excess liquid with a glass rod or photographic roller, heat to 80°C for 10 minutes in the thermostat.

As an alternative to the procedure in transparency, perform the drying and whitening method for Cellogel sheets to enhance the sensitivity and to be able to observe the barely visible peaks on the transilluminator.

**TWO-DIMENSIONAL IMMUNOELECTROPHORESIS:**
Unconcentrate Cerebrospinal Fluid, Unconcentrate Urine and other biological liquids with low protein concentrations

The same method is used as described for two-dimensional immunoelectrophoresis of serum protein with the following variations in the application of the specimen and of volume and type of anti-serum:
- specimen with total proteins lower than 500 mg/l: 4 deposits of 2 μl (8 μl in total). Antiserum: 30 μl + 120 μl saline solution.
- specimen with total proteins higher than 500 mg/l: 3 deposits of 2 μl or less in relation to the total proteins. Antiserum 40/50 μl + 110/100 μl saline solution.
- For cerebrospinal fluid use antiserum enriched with anti-prealbumin and anti-albumin (e.g. Dako A208).
- For urines use antiserum Dako A211.

Bibliography:
It is possible to transfer the proteins which migrated on Cellogel, immediately after high resolution electrophoresis onto a nitrocellulose membrane with porosity of 0.2 or even 0.4 microns with a very simple technique described by French authors (Cohen, Lambrey and Dropsy, J. Imm. Meth. 104, 25-30, 1987) which involves wringing the Cellogel strip coupled with a sheet of nitrocellulose between two rubber rollers. Place the Cellogel film on a well cleaned plate of glass, over it place a strip or sheet of nitrocellulose, add another sheet of dry fine grain filter paper. Passing a roller, of the type used in photographic laboratories, over it, the transfer is accomplished without any of the complications familiar to those who undertake Immunoblotting by transferring the migrations obtained on polyacrylamide gel onto a membrane. If Immobilon is used, this membrane is first treated with methanol and then buffered in PBS pH7.4 it is used when wet. The reason for the transfer of the migrations from Cellogel to the above mentioned membranes is due to the advantages obtained from the fact that all the proteins are precipitated onto the nitrocellulose membrane or onto Immobilon. The proteins, in fact, remain well displayed with their sites of antigen reagents on their surface, it become possible to use monoclonal antibodies unable to immunoprecipitate proteins contained in the migrations of gels.

For the identification, above all, of trace proteins (e.g. ferritin, CEA, β2 microglobulin, etc) and it becomes possible to apply the powerful systems of revelation of immuno-histochemistry to reveal quantities of picograms of proteic bands. In practice, the film of nitrocellulose or Immobilon, after the transfer has taken place, is immersed in a solution of PBS or TBS (Tris Buffered Saline pH 7.4) containing BSA 1% and mouse monoclonal antibodies diluted 1:100-1:1000. After incubation of at least 2 hours with gentle stirring the membrane is washed 2-3 times in PBS or TBS. This is followed by treatment with the “bridge antibody”, that is to say Rabbit anti-Mouse lgs (code 911CO) diluted 1:100 in TBS. After 30 minutes incubation wash the excess of Rabbit antibody. Incubate for 30 minutes in PAP Mouse Complex (code 650HE) diluted 1:100 in TBS. Wash plentifully in TBS (3 rinses in 15 minutes).

Prepare the AEC: 14 ml acetate buffer (code 888MA) + 1 ml AEC solution (code 925MA) + 150 μl H2O2 3% (code 889MA). Should the solution give a precipitate, filter it. Immerse the strip and incubate until the coloured bands appear. The monoclonal biotinilated diluted 1:100 in TBS solution containing 1% BSA system can also be used. After washing treat with Streptavidin HRP and then stain with AEC of the peroxidase.

MOST-EIA MEMBRANE ORDINATE SPOT TEST – ENZIMO IMMUNO ASSAY

This is the immunoenzymatic method on membrane and can be applied for all the analytes of serum and other biological fluids which can be tested on ELISA microplate. The membrane utilised measures 5.7x7 cm and is made of Cellogel C18 which has the characteristic of retaining the proteins via hydrophobic interaction (well known to experts of HPLC Chromatography).
Because the membrane is filtering and the hydrophobic properties are reversible according to their salinity, it is possible to avoid the primary capture antibody retaining the analyte (antigen), looked for, wash it and make it react with a non-precipitating marked monoclonal antibody (e.g. with Biotin).

The washes of the Cellogel C18 membrane are performed in times reduced to 2-3 minutes on Filtrawasher (code 13A44) with a few ml of TBS or PBS. After treating with the biotinilate monoclonal and washing, immunovisualization is performed with incubation in Streptavidin HRP followed by washing in TBS and treatment in a bath of AEC. The membrane can be cleared and read with Glob Al Scan for rapid quantitative determination of the desired analyte. As in the case of MOST-ICA it is possible to perform 42 tests for the antigen desired (e.g. Tumour marker) + 6 calibration points. The international patent on MOST-EIA is valid for years and it is therefore only supplied to order under the relative distribution licence.

IMMUNOVISUALIZATION OF ANTIGENS USING NON-BIOTINILATE MONOCLONALS ON CELLOGEL C18 IMMUNODOT MEMBRANE

The MOST-EIA technology of Cellogel-C18 (amphiphilic) also foresees the use of non-biotinilate monoclonals. In this case the use of the anti-mouse antibody bridge and PAP technique with staining of the peroxidase with Chromogen AEC is necessary.
15. CELLOGEL/MYL FOR ELECTROPHORESIS, HORIZONTAL AND ARCHED

Cellogel/Myl is a wet Cellogel membrane supported on Mylar. Cellogel/Myl was produced to satisfy the requests from laboratories using the Helena system of “horizontal electrophoresis” on dry acetate. This can, in fact, substitute Titan III in Zip Zone chambers with the advantage of practicality and the possibility to perform high resolution electrophoresis. Cellogel/Myl facilitates the discovery of monoclonal bands because it permits the lengthening of the gamma zone much more than agarose and other known gels, thanks to the strong electroendosmosis with retromigration of the immunoglobulins. It is, therefore, suitable not only for high resolution electrophoresis but also for high definition electrophoresis as shown in the example illustrated where the gamma zone extends for about 7 cm with the polyclonal immunoglobulins reduced to a minimum and the small monoclonals compacted and enhanced to the maximum.

High Definition Electrophoresis on Cellogel/Myl is an advanced system of High Resolution for serum protein, urine, cerebrospinal fluid, etc. proposed by Cellogel Co. for the clinical identification of difficult cases of monoclonal gammapathies and also for the identification of the protein “Minor Components” of serum. With this method 20-30 bands can be revealed with only 0.9 μl of specimen. Two procedures are proposed for immunovisualization of the bands:
1. Immunofixation with specific polyclonal antibodies against Minor Components in quantities of some tens of ng.
2. Rollblotting with immediate transfer of all the separated components onto a membrane of nitrocellulose by the method of Cohen, Lambrey and Dropsy (J. Immun. Meth. 104, 25-30, 1987). The nitrocellulose membrane is subsequently incubated with mixtures of specific monoclonal antibodies in a 1% BSA solution (blocking).
With this procedure the Minor Components present in quantities of a few picograms can be identified. Immunovisualization is performed with the system of Streptavidin biotin HRP (code 926 MA) suitable for revealing antibodies, both monoclonal and polyclonal of rabbit. It is possible to reveal tumour markers as, for example, CEA, Ferritin, AFP, PSA, β2-micro etc.

**Materials necessary for HD Electrophoresis:** Power Supply 0-500V; electrophoresis chamber; semi-micro Applicator for 4 deposits (code 12A17-4P3).

**Reagents necessary:** Cellogel/Myl 7x23 cm (code 01H16-10); TGS buffer (code 02A03-10); Coomassie Stain BB 250 R; Super Gold Stain (code 23G05); Drying whitener (code 07A02-S).

**Materials necessary for immuno-staining of some tumour markers:** NC Membrane 6x16 cm; Streptavidin-HRP/Biotin Kit code 926 MA; Monoclonal Antibodies AFP Mab (IgG 1K) code 766 OC; CEA Mab (IgG1) code 767 OC; Ferritin Mab (IgG 1) code 684 OC; Polyclonal Antibodies (Rabbit Igs) β2 microglobulin code 19R39; RBP Retinol Binding Protein code 19R55; TBG Thyroxine Binding Globulin code 19R57; PSA code 35B03.

**CELLOGEL/MYL SIZES**

- 6x7.6 cm (cod. 01H01-10) ) Helena size for Zip Zone chambers and relative applicators.

- 5.7x7.7 cm (cod. 01H01-B-10) for Cellogel/Myl tank (code 11A11/B) or Helena tank and semi-micro applicators for 4 deposits (code 12A17-4P3) or micro for 8 (code 12A16).

- 19.85x7.6 cm (cod. 01H18-10) for horizontal electrophoresis of 18 specimens with Cellogel/Myl (code 11A11/B) or Helena tanks and applicator for 18 deposits (code 12A20-CM).

- 7x23 cm (cod. 01H16-10) for arched Electrophoresis with Cellogel/Myl tanks (code 11A11/B).

- 5.7x10.2 cm (cod. 01H06-10) for arched Electrophoresis with Cellogel/Myl tanks (code 11A11/B).

- 10.2x7.6 cm (cod. 01H03-10) Beckman size with 18 deposit applicator (code 12A20-CM).

- 11.4x13.7 cm (cod. 01H04-10) size for Corning tank and 18 deposit applicator (code 12A20-CM).

- 23.5x7 cm (cod. 01H19-10) for automatic equipment, EPU type.

*All the packets contain 10 individually-wrapped films. Other sizes are available on request.*
16. EQUIPMENT FOR CELLOGEL/MYL

POWER SUPPLY

**Code 10A08/B**
The Power Pack 300 V is suitable for all kinds of electrophoresis which require up to 300 V. The digital display indicates the voltage values (which can be constantly regulated) and the current. The two exit points mean that two tanks can be supplied simultaneously.

- **Voltage:** 0-300 V DC current
- **Amperage:** 0-100 mA
- **Exit Points:** 2 (for 2 tanks)
- **Timer:** 3s - 60h
- **Input:** 220V (on request 110 V) 50/60 Hz
- **Dimensions:** 27x21x11 cm
- **Weight:** 3.8 Kg

CHAMBER FOR CELLOGEL/MYL  (UNIVERSAL CHAMBER)

**Code 11A11/B**
Chamber, for arched and horizontal electrophoresis, on Cellogel/Myl or commercial Agarose film. Produced both for routine and research uses. Polycarbonate injection moulded tank with high chemical and physical resistance. Smoky-grey semi-transparent polycarbonate lid with two magnets for safety micro-switch and interruption of the current when the lid is opened. Platinum electrodes, 19 cm long. A bridge for horizontal electrophoresis for 3 films (5.7x7.7 cm) or one film (19.85x7.6 cm) for use with the multiple applicator for 18 deposits (code 12A20-CM); a bridge for arched electrophoresis for 5.7x10.2 cm film and for Agarose film in the formats Beckman, Sebia or Helena.

- **Dimensions:** base 25 x 19 cm, height 5 cm
- **Capacity:** 200 ml of buffer solution per compartment
- **Weight (including 2 bridges):** 1 Kg
BRIDGES FOR CELLOGEL/MYL

Bridge for horizontal electrophoresis, 7.6 cm code 11B20
Bridge for arched electrophoresis code 11B19

APPLICATORS FOR CELLOGEL/MYL

a - Micro Applicator for 8 specimens code 12A16
b - Semi-micro Applicator for 4 specimens code 12A17-4P3
c - Semi-micro Applicator for 18 specimens code 12A20-CM

EXAMPLE OF MICRO ELECTROPHORESIS OF THE SERUM PROTEIN IN HORIZONTAL POSITION OF CELLOGEL/MYL FILM (5.7X7.7 cm) USING A UNIVERSAL TANK (CODE 11A11/B) OR A HELENA ZIP ZONE TANK:

1. Buffer the film in Tris Hippurate (code 02C13-2X-6) for 10 minutes in minibox (code 13A51) on rotating shaker; 20 ml of buffer solution are sufficient. Horizontal buffering gives rise to savings of large volumes of buffer. The same can be said of staining and destaining. During buffering pour the buffer solution into the tank (200 ml per compartment), wet the paper wicks (code 13B19) in the tank and place it astride the bridge. Cellogel/Myl has the advantage that it can be immersed directly into the buffer as it is already wet and does not present the problem of entrapping air in its pores as occurs with dry acetate which is normally buffered vertically.

2. Pipette 20 µl of serum on each drop holder of the specimen holder base. Check that the applicator is perfectly clean.
3. Remove excess buffer solution between 2 sheets of filter paper and place the film on the base of the applicator. Apply the specimens at about 3.5 cm from the cathodic edge for 10 seconds.

4. Position the film in the tank with the gel facing downwards and the deposit zone at the negative pole. Ascertain that contact between the film and the paper wicks of the bridge is perfect.

5. Plug the tank into the power supply, cover the tank and apply 160 V for 18 minutes to obtain 22 mm migration.

6. After electrophoresis, switch off the power supply. Extract the strip and immerse it in a bath of Ponceau S for 5 minutes. Return the stain to its bottle. Destain in acetic acid 5% (or 3% odourless citric acid) for 3 baths of 3-4 minutes each.

Clearing: immerse in a bath of clearing solution (code 06A06-S1) for 5 minutes. Remove excess liquid with a glass rod and heat in thermostat at 80°C for 10 minutes. After cooling for 1 minute at room temperature the film is placed on the scanner for the densitometric reading.

With Cellogel/Myl it is possible to perform electrophoresis for research, immunological techniques, such as Ouchterlony Technique and the Mancini Technique of Quantitative Radial Immunodiffusion.

### CELLOGEL/MYL 6x7.6 cm and 5.7x7.7 cm Horizontal Electrophoresis – WALL CHART

Clinical Electrophoresis micro methods with:
- Helena Zip Zone System and Cellogel/Myl 6x7.6 cm
- Cellogel Universal chamber and Cellogel/Myl 5.7x7.7 cm

<table>
<thead>
<tr>
<th>SERUM PROTEINS</th>
<th>SERUM PROTEINS</th>
<th>UNCONCENTRATED URINE PROTEINS</th>
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</tr>
<tr>
<td>Tris-Hippurate</td>
<td>Tris Glycine Salicylic Acid (TGS)</td>
<td>Tris Glycine Salicylic Acid (TGS)</td>
</tr>
<tr>
<td>10 min. equilibration in a small box with 40 ml buffer</td>
<td>10 min. equilibration in a small box with 40 ml buffer</td>
<td>10 min. equilibration in a small box with 40 ml buffer</td>
</tr>
<tr>
<td>Sample</td>
<td>Sample</td>
<td>Sample</td>
</tr>
<tr>
<td>micro 0.3 µl /4 mm</td>
<td>micro 0.3 µl /4 mm</td>
<td>micro 0.3 µl /4 mm</td>
</tr>
<tr>
<td>Start Point</td>
<td>Start Point</td>
<td>Start Point</td>
</tr>
<tr>
<td>3.5 cm from cathodic (-) edge</td>
<td>3.5 cm from cathodic (-) edge</td>
<td>3.5 cm from cathodic (-) edge</td>
</tr>
<tr>
<td>Voltage</td>
<td>Voltage</td>
<td>Voltage</td>
</tr>
<tr>
<td>160 V</td>
<td>200 V</td>
<td>200 V</td>
</tr>
<tr>
<td>Time</td>
<td>Time</td>
<td>Time</td>
</tr>
<tr>
<td>18 min.</td>
<td>20 min.</td>
<td>20 min.</td>
</tr>
<tr>
<td>Stain</td>
<td>Stain</td>
<td>Fix</td>
</tr>
<tr>
<td>7 min. immersion in Ponceau S (0.5 g in 100 ml of 5% Trichloracetic Acid)</td>
<td>7 min. immersion in Amidoblack (0.3 g in 100 ml of 20% Acetic Acid)</td>
<td>in 10% Trichloracetic Acid contained in Silver Stain Kit</td>
</tr>
<tr>
<td>Destain</td>
<td>Destain</td>
<td>Wash</td>
</tr>
<tr>
<td>3 baths of 5% Acetic Acid</td>
<td>3 baths of destaining solution (475 ml Methanol + 475 ml H2O + 50 ml Acetic Acid)</td>
<td>in 3 baths of H2O</td>
</tr>
<tr>
<td>Clear</td>
<td>Clear</td>
<td>Stain</td>
</tr>
<tr>
<td>Immerse for 5 minutes in a clearing solution (code 06A06-S1) bath of 40 ml. Warm at 80°C in the oven for 10 minutes</td>
<td>Let to dry destained film at open air for 15 min.</td>
<td>9 min. in Silver Blue Stain Kit</td>
</tr>
<tr>
<td>Read and record</td>
<td>Wash</td>
<td>Stain</td>
</tr>
<tr>
<td>at Glob-Al Scan</td>
<td>at transilluminator (in dark room)</td>
<td>at Glob-Al Scan (before drying process)</td>
</tr>
<tr>
<td>Print the report and record</td>
<td>presence of MC</td>
<td>Immerse in 20-30 second in Blue toner contained in the Silver Stain Kit</td>
</tr>
<tr>
<td>as for Serum Protein</td>
<td>Blue Toner</td>
<td>as for Serum Protein</td>
</tr>
<tr>
<td>Read and record</td>
<td>at Glob-Al Scan</td>
<td>at Glob-Al Scan</td>
</tr>
</tbody>
</table>
The new product Celloclear Agarose Plus surpasses all the other technologies of Capillary electrophoresis and electrophoresis on agarose both for speed and diagnostic reliability. With an 18x24 cm film it is possible to carry out up to 144 tests of serum proteins in 1 hour, 54 High Resolution tests of serum proteins in 1 hour and 18 IFE tests in 2 hours.

Electrophoresis on Celloclear Agarose Plus is very simple and much easier than on agarose; the costly systems of circulating cold water or Peltier control which are necessary with all the commercial agarose gels are not necessary.

For the daily routine of large public of private laboratories Celloclear Agarose Plus is the most valid system, rapid and economical, existing on the international market for the identification of asymptomatic monoclonal gammapathies.

*The Celloclear Agarose Clear catalogue will be supplied on request.*