IEFGel 6-11
Manual

V7 11/17

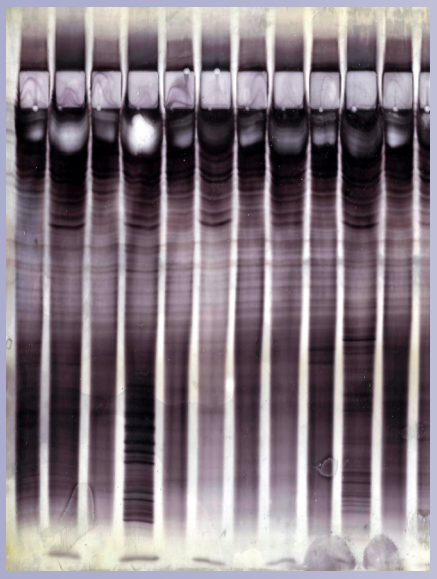


Fig.A:
Ammoniacal Silver-Staining

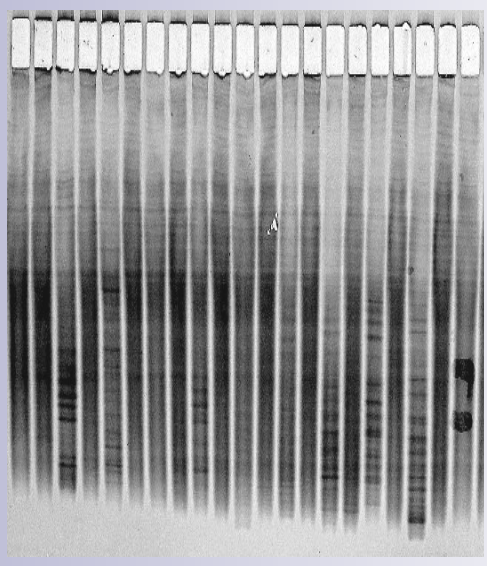


Fig.B:
Immuno-Fluorescence

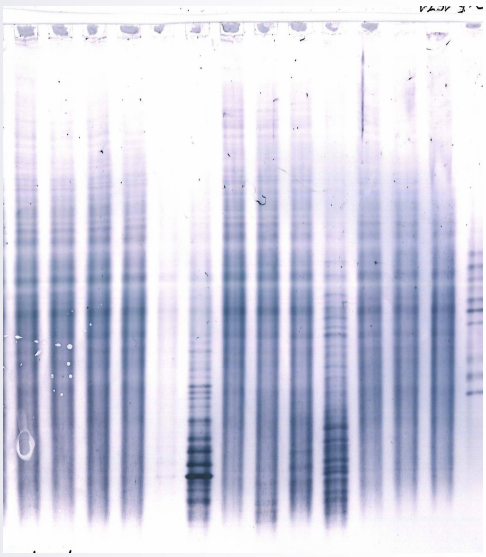


Fig.C:
Immuno-Staining

***General**: IEFGels are 0.65 mm thin polyacrylamide gels with T = 5% gel concentration and C = 3% crosslinking for isoelectric focusing, which have been polymerized under special conditions, in order to obtain an optimal matrix suitable for isoelectric focusing.*
Catalysts, other toxic and non polymerized substances like acrylamide monomers have been removed from the matrix. Therefore IEFGels are not toxic.The gels contain a special carrier ampholyte cocktail to achieve an optimal pH 6- 11 gradient. The samples are applied into preformed sample wells (slots).

Three visualization techniques are described:

- **A** *Ammoniacal Silver-Staining (Fig.A) as general protein staining*
- **B1** *Immuno-Fluorescence (Fig.B)*
- **B2** *Immunofixation followed by Silver Staining*
- **C** *Blotting procedure with Immuno-Staining (Fig.C).*

Gels IEFGel 6-11 24S (edc-1001): 4 gels for native IEF, 0.65 mm, 24 slots à 20µl
IEFGel 6-11 40S (edc-1002): 4 gels for native IEF, 0.65 mm, 40 slots à 10µl
IEFGel 6-11 80S (edc-1003): 4 gels for native IEF, 0.65 mm, 80 slots à 10µl

Additionally required

DryPool Combi (*edc-me-d*)
Tray for rehydration of dry gels (normal size)
and soaking electrode strips

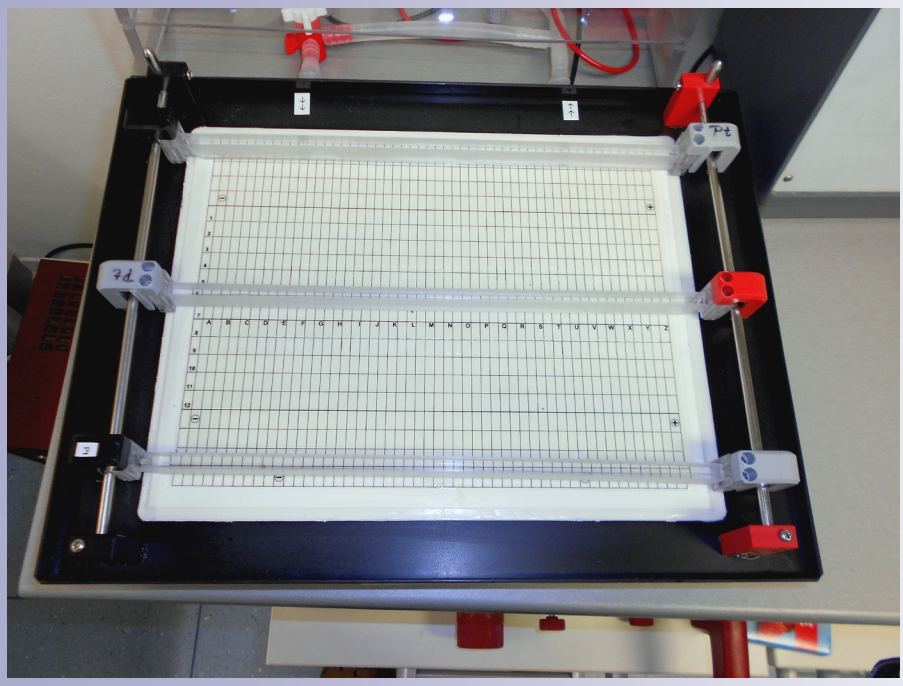
Consumables:

IgG Serum Diluter: blue colour, 0.1% NaCl;250 ml (edc-2001)
IgG CSF Diluter: yellow colour, no NaCl; 100 ml (edc-2001)

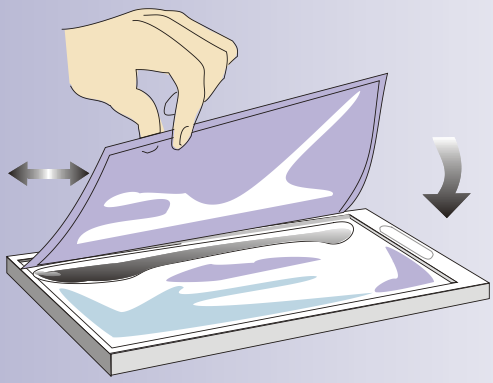
Goat anti-human IgG (H+L) / T-REX, special conjugated antibody for Immunofluorescence, for 4 gels; (edc-2020) 1.2 ml

Chemicals
Kerosene (Serva 26940)
Silver-staining: Trichloroacetic acid (Merck 1.00807), methanol (Merck 8.22283), ethanol (local dealer), acetic acid (local dealer), glutardialdehyde (Merck 8.20603), formaldehyde (Merck 1.04003), sodium acetate (Merck 1.06267), ammonia solution (Merck 1.05432), citric acid (Merck 1.00244), $AgNO_3$ (Merck 1.01512)
Immuno-staining: Nitrocellulose 0.45 µm pore-size (S & S, Protran BA 85), Whatman´s Paper No.1, skim milk powder (BioRad), goat anti-human IgG-Fc (MP-products #151083707), rabbit anti-goat IgG HRP-conjugated (DAKO #P0449), ethyl-aminocarbazole (Sigma #A5754)
Immuno-fluorescence: Polyclonal Rabbit Anti-Human IgG/FITC (fluorescence conjugated antibody, DAKO F020). Non-fluorescence Immuno-fixation: Anti human IgG (Dako #A0424), Rabbit Anti Human Transferrin (Dako #A0061), polyethylene glycol 6000 (Merck 817007).

Equipment
Power supply 2 kV, Chambers: flatbed IEF professional (edc-IEF-2836) or flatbed basic (edc-FB-2836) or Multiphor II (GE 18-1018-06).
Thermostatic Circulator.
Immunofluorescence: GE Healthcare Typhoon: (9200), Fuji: FLA 7000, 5100. Rehydration Tray for all immuno reactions (edc-ME-S for normal gels, edc-ME-L for bidirectional gels).



flatbed IEF professional
with 3 electrodes



DryPool Combi
used for the Immuno
reactions

Unpackinge the IEFGel

Open the bag with scissors, see Fig. 1. Then carefully remove the cover-film from the gel surface. Do not throw it away, it will serve as a preserving sheet later! The gel is now ready to use!

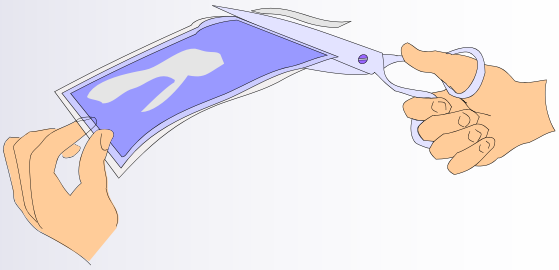


Fig. 1: Cutting the plastic bag

Sample Treatment

All dilutions (incl.the 1:100 dilution-step of the sera) must be performed with the IgG Serum Diluter (or 0.1% NaCl), res. the IgG CSF-Diluter (or H_2O dist., no NaCl), do not use PBS!*) The IgG-concentrations are determined with a nephelometer! Sample volume: 20 µl (24S), res. 10 µl (40S).

*)Phosphate-Buffered Saline (0.9%)

Sample dilution, Serum and Cerebrospinal Fluid:

All sera should first be diluted: 5 µl Serum + 495 µl IgG Serum Diluter (or 0.1%NaCl). In the case of general silver staining (visualization A) or blotting with immunostain (visualization C) all sera are adjusted to 20 mg/l IgG. If other visualizations are used, please see the special IgG-concentrations described in the applied visualization.

All cerebrospinal fluids are directly adjusted to the same protein concentration like the sera using the IgG-CSF Diluter (or H_2O dist., no NaCl).

Setting the Gel onto the Cooling Plate

The cooling plate should not be cooled unless the samples are pipetted and the lid is closed: Otherwise water condensation on the gel-surface draw the samples out of the slots!

A socalled „Cooling Bypass" can be used to cool down only the cooling apparatus, fig 1b. Spread 2.5 ml (res. 4 ml for a bidirect gel) of kerosene onto the cooling plate of the focusing chamber to ensure good cooling contact (Fig. 2). Place the gel (surface up) onto the center of the cooling plate (Fig. 3). The wells should be orientated towards the anode. Avoid trapping of air bubbles.

Multiphor II:The cathodal edges of the wells should be aligned along line 11 of the cooling plate.

Exceed volume of kerosene around the gel has to be absorbed by a cosmetic tissue.

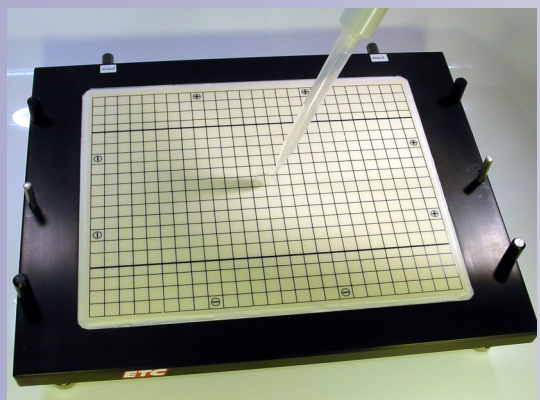


Fig. 2: Applying kerosene onto the cooling plate (flatbed basic)

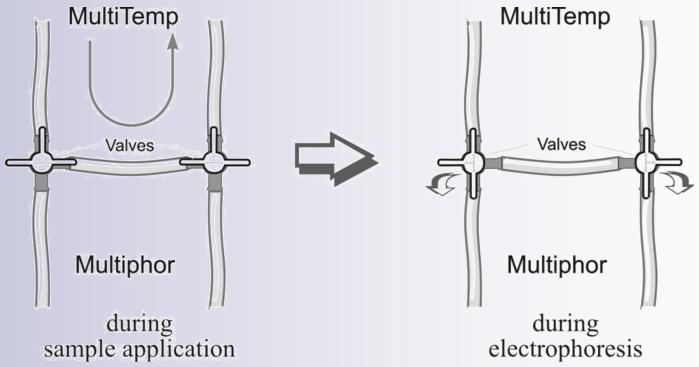


Fig. 1b: The „Cooling Bypass" system to cool only the kryostate while sample application

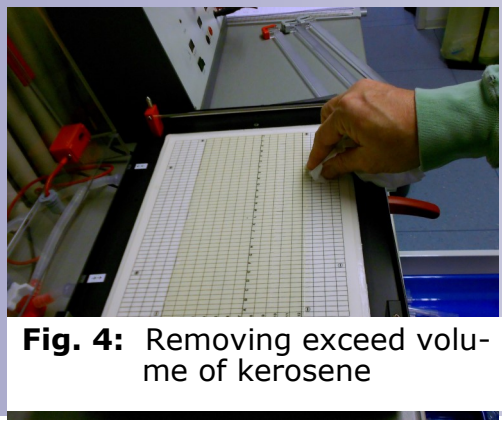


Fig. 4: Removing exceed volume of kerosene

Fig. 3: Placing the gel onto the cooling plate (flatbed IEF professional)

Using flatbed IEF Professional: Electrodes Application
Using the flatbed IEF professional the electrodes are applied before the sample application.
Take the anode with the red head orientated to the anodal red marked positive electrode bar (right side of the chamber) and snap it down to its final position. Figure 8 and 9.

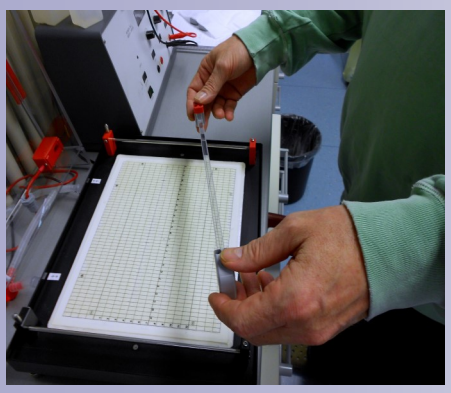


Fig.8: The red anode goes to the right, red marked electrode bar*

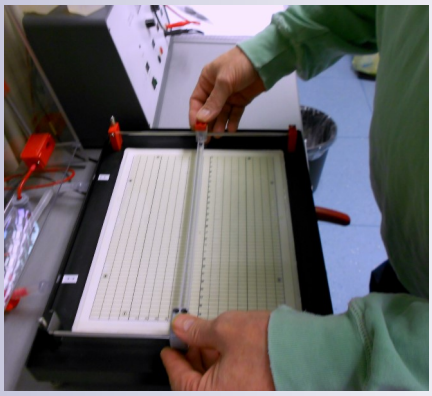


Fig.9: The anode is pressed down to its final position*

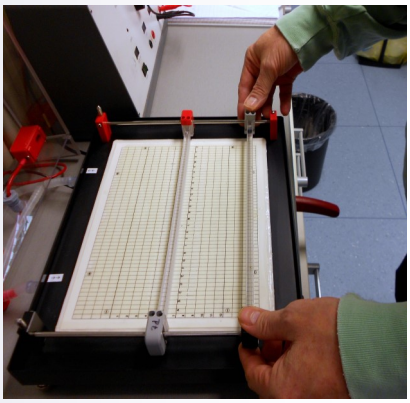


Fig.10: The black cathode goes to the black marked electrode bar*

The black headed cathode has to be orientated on the black marked negative electrode bar. After this electrode is pressed down to its final position, exceed volume of the cooling solution will be pressed out between gel and cooling plate. This could be absorbed by a cosmetic tissue.
Do this at both electrodal sides. Figure 11.

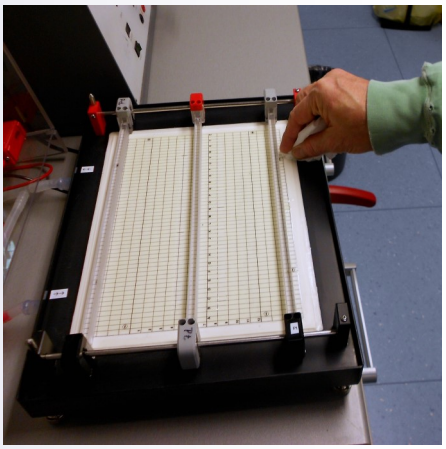
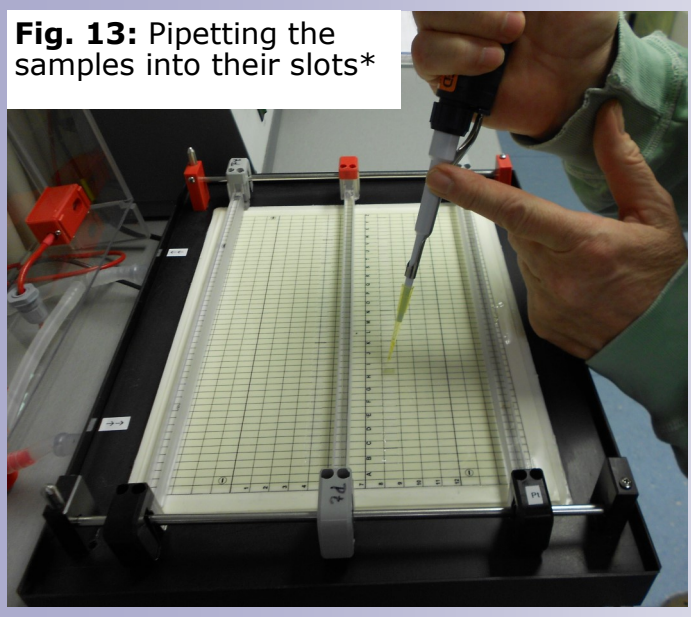


Fig. 11: Exceed cooling fluid is absorbed by a cosmetic tissue*

Using all other chambers: Sample Application
Using all other types of electrophoresis chambers the electrodes (normally built in the security lid) are set on the gel‘s surface after samples are applied
Sample wells filling: Apply 20 µl (24S) or 10 µl (40S, 2 x 40S) of serum and cerebrospinal fluid alternating, see figure 12. Do not leave any slots unfilled. Apply 20 µl of the Sample Diluent to non-used slots. Figure 13.

Fig. 13: Pipetting the samples into their slots*



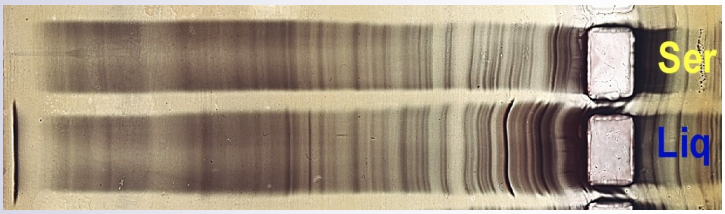


Fig. 12: For a better comparison CSF and Serum samples are applied alternativly

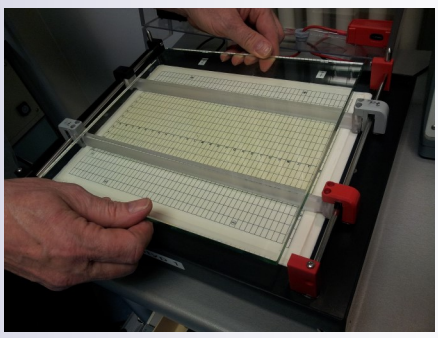


Fig. 14: IEF-professional**:** Placing the Heavy Weight glasplate on the electrodes

For all other chambers: Electrode Application
Application of the Electrodes (normal gels): Clean the 2 platinum wires with wet tissue papers before (and after) IEF thoroughly. Move the platinum electrodes to their positions over the edges of the gel. flatbed basic: Lower the electrode holder onto the gel surface. Connect the electrode cables to the plugs in the chamber, close safety lid and begin with focusing (Table 1). flatbed IEF professional: Snap the electrodes down on the gel's surface and close the safety lid.

Temperature
Isoelectric focusing has to be performed at a defined constant temperature, because the pH gradient and the isoelectric points depend on the temperature. Switch on the thermostatic circulator, set to 7 °C and do not forget to switch the Cooling Bypass to position „electrophoresis" (fig. 1b) if this technique is used.

Flatbed IEF professional: Place the heavy-weight glasplate on the electrodes and close the safety lid. Remove squeezed-out kerosene by the edge of a kitchen paper. Do this at both electrodal sides. Figure 14.

Running Conditions
During isoelectric focusing the electric resistance of the gel is changing considerably; in the end phase low current - and power values - determine the voltage values of the run. The most commonly used method is to limit the voltage via the mA and the Watts achieved in the gel during the run. The Volt, mA and Watt values given in table 1 should be applied; in case of bidirectional runs table 2 is used.
The three different phases must be run in a direct sequence! Ideally a programmable power supply is used.
When the red dye leaves the slot in anodal direction, the gel was orientated correctly!

Do not use the mode „Gradient" offered by some more modern power supplies!

Tab. 1 normal gels (25 x 11 cm): Run limited by mA and Watt (Comm. used method)

Note 1: In the beginning of each single step the starting volt-value (in italics and in parenthesis) should be adjusted via the limiting mA or W values.

Table 1	SET	*Start Value*	SET	SET	Time	Process
Step 1	500 V	*(270 V)*	25 mA	10 W	20 min	sample entrance
Step 2	1350 V	*(650 V)*	25 mA	22 W	90 min	main focusing
Step 3	1750 V	*(1750 V)*	20 mA	26 W	20 min	band sharpening

Tab. 2 bidirectional gels (18 x 25 cm) 2 peripheral cathodes and 1 central ano-

Table 2	SET	*Start Value*	SET	SET	Time	Process
Step 1	450 V	*(220 V)*	45 mA	15 W	15 min	sample entrance
Step 2	900 V	*(570 V)*	35 mA	40 W	80 min	main focusing
Step 3	1300 V	*(1300 V)*	30 mA	40 W	15 min	band sharpening

BioRad Power supply: Do not take the "IEF"-method, select "Basic". The value set to constant have a darker background colour in the display.

These running conditions are only valid for aluminum oxid ceramics cooling plates!
If the cooling plates are made of metal and/or glass: Do not apply more than 10 W!
After IEF: Immediately fix the gel with TCA or antibody solution, or perform blotting.

The Visualization Procedures
A Ammoniacal Silver Staining: All proteins are stained. Best resolution, but not IgG-selective.
B1 Immunofixation with Fluorescence Imaging: IgG-selective staining, very sensitive. Needs a Fluorescence Imager.
B2 Immunofixation followed by Silver Staining: IgG-selective staining, very sensitive. Needs an overnight step (washing out of the excess antibodies).
C Blotting with Immuno-Staining: IgG-selective staining, needs an additional blotting step.

Proposed Strategy: Method A gives best resolution and sensitivity. This method should be taken as routine method.
Critical samples should be collected and then run with an IgG-selective visualization, such as B1, B2 or C.


Fig. 14: GE's Hoefer Processor Plus

A General Silver-Staining: Solutions
Silver staining can be automated with a Processor Plus from Hoefer (see Fig. 14).
Adapted from reference [1]
Attention: The new IEFGels will bob up sometimes in the Autostainer! *Fix these gels with the magnetic bars coming with the staining mashine.*
Before the staining: To remove protein from the gel-surface and kerosene from the support-film wash the gel with hot water; then with distilled water acc. to fig 14b.

1 Fixing : *30g TCA + 110 ml H_2O dist.+ 60 ml MeOH = 200 ml*
Attention: *Fixing solutions containing TCA and MeOH have to be prepared freshly!*
2 Rinsing I: *100 ml MeOH + 20 ml HAc, fill up to 200 ml*
3 Rinsing II: *10 ml MeOH + 14 ml HAc, fill up to 200 ml*
4 Incubating : *20 ml Glutaraldidehyde (25%), fill up to 200 ml with H_2O dist. then add 3.5g NaAc*
9 Water : *1400 ml H_2O dist.*
6 Silvering : *Solution 1: Dissolve 250 mg $AgNO_3$ in 1 ml H_2O dist.*
Solution 2: 40 ml H_20 dist + 4 ml NaOH (1M) + 1.5 ml NH_3 (25%)
Drop Solution 1 into 2 while stirring, fill up to 200 ml
with dist H_2O, (or see the Silver-Staining Kit manual)
7 Developing : *5 mg citric acid + 100 µl formaldehyde, fill up to 200 ml*
with H_2O dist, (or see the Silver-Staining Kit manual)
8 Stopping : *20 ml EtOH + 2 ml HAc*
fill up to 200 ml with H_2O dist.
9. Preserving *:* *10 ml glycerol + 190 ml H_2O dist.*


Fig. 14b: Washing the gel's surface and the film support

Abbreviations:
HAc (Acetic acid), NaAc (Sodium Acetate), MeOH (Methanol), EtOH (Ethanol), TCA (Trichloroacetic acid), MeOH (Methanol)
References:
[1] Rabilloud T: A comparison between low background silver diamine and silber nitrate protein stains. Electrophoresis (1992) 13, 429 - 439

General Silver-Staining: Solutions and Programme

Do not use plastic ware: glas or stainless steel ware!

Step	Solution	Vol.	gel<0.5mm	gel>0.5mm
1. Fixing	15% trichloroacetic acid (w/v) 30% Methanol	200 ml	20 min	30 min
2. Washing	H_2O dist	200 ml	5 min	5 min
3. Rinsing I	50 % methanol / 10% acetic acid (v/v)	200 ml	20 min	30 min
4. Rinsing II	5% methanol / 7% acetic acid	200 ml	15 min	20 min
5. Incubation	2.5 % glutardialdehyde	200 ml	15 min	20 min
6 - 9. Washing	H_2O dist (optional: hold overnight in the last step)	3 (4) × 200 ml	3 x 10 min	3 x 15 min
10. Silvering *(Prepare freshly before use!)*	Solution 1: Dissolve 250 mg $AgNO_3$ in 1 ml H_20 dist. Solution 2: 40 ml H_20 dist + 4 ml NaOH (1M) + 1.5 ml NH_3 (25%) Drop Solution 1 into 2 while stirring, fill up to 200 ml with dist H_2O *(Or see the Silver-Staining Kit manual)*	200 ml	25 min	30 min
11-12. Washing	H_2O dist	2 × 200 ml	1 + 5 min	1 + 5 min
13. Developing *(Prepare freshly before use!)*	0.0025% citric acid + 100 µl formaldehyde in 200 ml with H_2O dist *(Or see the Silver-Staining Kit manual)*	200 ml	1-4 min Set Beep: Step 12 Watch and stop when background turns yellow!	1-4 min Set Beep: Step 12 Watch and stop when background turns yellow!
14. Stopping	10% ethanol, 1% acetic acid	200 ml	15 min	20 min
15. Preserving	5% glycerol	200 ml	15 min	20 min

Drying: Air-dry the gel (down to the film), then roll on the polyester cover sheet (supplied with the gel) onto the surface.

Total staining time: Approximately 3.5 hours (gel-thickness < 0.5mm); *4 hours (gel-thickness* > 0.5mm*).*

Stock Solutions and more detailed mixing recipe, please see:
http://www.electrophoresis-development-consulting.de

B1 Immuno-Fixation with Fluorescence Imaging

Before the staining: To remove protein from the gel-surface and kerosene from the support-film wash the gel with hot water; then with distilled water acc. to fig 14b.

All samples are adjusted to 15 mg/l IgG. The Immunofixation can be done in a flat tray, best is the use of the Rehydration Tray. The tray should stand on an orbital shaker. Pipet the 30 ml antibody solution into the tray and lay the gel, gel-side down, onto the surface of the solution. Switch on the shaker; select the slowest setting. See Fig. 15–17. The gel must not stick to the tray's bottom!
Do not write on the plastic film!

Step	Solution	Volume	Temp	Time
Fluorescence Immunofixation	300 µl Goat anti-human IgG (H+L) / T-REX in 30 ml 3 % PEG 6000 in PBS*)	30 ml rocked!	20°C	2 h
Washing	3 % PEG 6000 in PBS*), rocked!	200 ml	20 °C	1 h

*)Preparing PBS (**P**hosphate **B**uffered **S**aline):
a) Phosphate Buffer: 2.5 g Na_2HPO_4 + 1 g NaH_2PO_4, fill up to 100 ml (= 320 mM). Gives pH 7.0.
b) PBS: 3.125 ml Phosphate Buffer + 810 mg NaCl + 20 mg KCl, fill up to 100 ml
(= 0.81% NaCl, 0.02% KCl in 10 mM buffer)

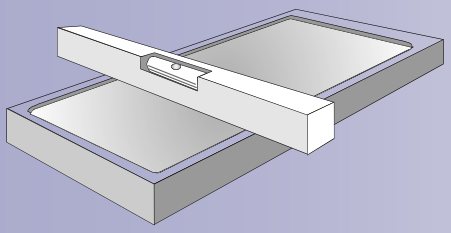


Fig. 15: EDC Rehydrati-onnTray, horizontally level-led

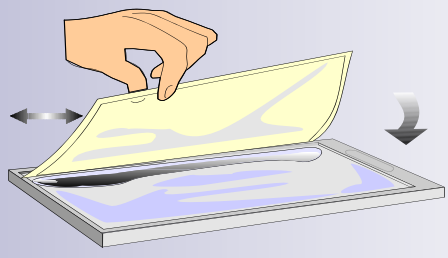


Fig. 16:.The gel is placed on the 30 ml antibody solu-tion

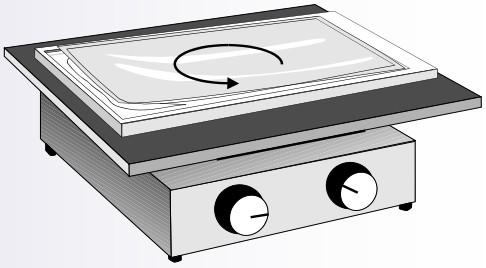


Fig. 17: The Rehydration Tray on an orbital shaker

Remove the gel from the washing solution. Place the wet gel, gel-side down, onto the scanner platen.
Scanner-Settings (GE and Fuji):
The T-REX conjugated antibody: physical properties: 648 nm excitation, 663 nm emissi-on.
The fluorescence-imager settings:
GE Healthcare / Fuji-scanner FLA9000: red laser, resolution: 25 µm
exciting: red LD Laser (635 nm), emission: LPR Filter (665LP)

B2 Immuno-Fixation followed by Silver-Staining

(Courtesy of G.Cox, Morriston Hospital Swansea)
Adjust the CSF- and the serum-samples to an IgG-concentration of 5 mg/l.

Step	Solution	Volume	Temp	Time
Immuno-Fixation	250 µl Rabbit Anti human IgG + 100 µl Rab-bit Anti-human Transferrin in 30 ml 3 % PEG 6000 in PBS*) , rocked!	30 ml	20 °C	2–3 h
Washing	3 % PEG 6000 in PBS*), rocked!	1000 ml	20 °C	overnight

Start silver-staining with 20 min H_2O dist., then from step 5 (Incubation), page 7

C Immuno-Blotting

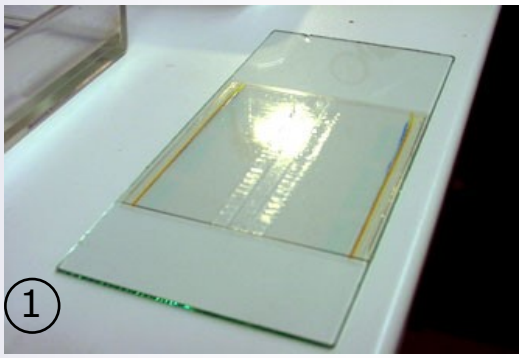
For Immuno-Staining the proteins should first be transferred to a blotting membrane.

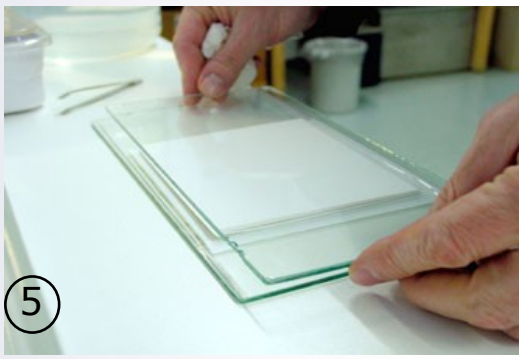
The Contact Blot:
The most simple blotting method after IEF is the "Contact Blot" (fig. 8). Using this transfer-method the plastic-support of the IEFGel has not to be removed. Because water must pass the membrane, a hydrophilic material is recommended: Nitrocellulose, 0.45 µm pore size. Alternative: PVDF-membranes: Mechanically more stable, but they have to be activated in Methanol first. Cut the required Whatman #1 filter papers and the blotting membrane to the size of the gel: 25.5 × 11.5 cm, res. 25.5 x 19 cm.

Important: After IEF first the gel's surface and the support has to be cleaned from non focused proteins: Hold the gel vertically and rinse the gel's surface and the support film thoroughly with hot water, then with dist.water (this will also remove the exceed kerosene volume), fig .14b.
After this dry the gel and the support film carefully with the edge of a filter paper.

Then the gel is placed onto a glass plate with the support-film (now cleand and dried) down (1) and the blotting membrane is directly placed onto the gel-surface (2) (now cleand and dried).
The air bubbles are pressed out with the help of a roller (3), starting from the midpoint to the four edges.
With the help of 5 filter papers (4), an additional glass plate (5) and a 1 kg weight (6) the proteins are forced to migrate onto the membrane.
Contact blotting time: 45 minutes.

Method (D.Zeman):
https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3306208/





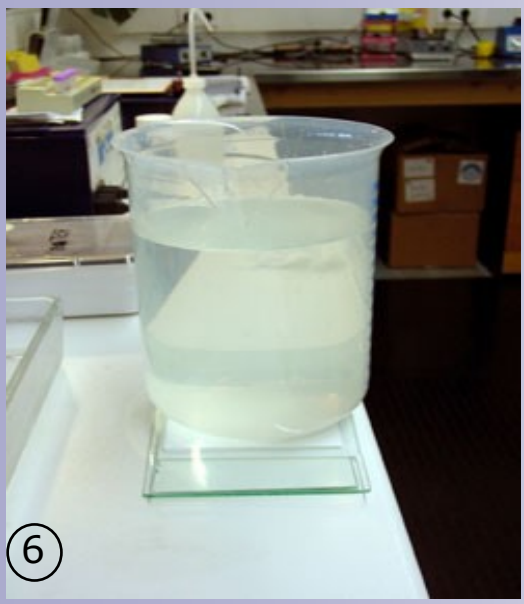


Fig. 8: The steps in Contact Blotting

Fig. 14b: Washing the gel's surface and the film support

The Immuno-Staining:
Place the membrane protein-side up in a clean glass tray and apply the following procedure:

Tab. 3: *General visualization programme for the blotting membrane (Courtesy of .A.W. Teelken UMCG, Neurologic Clinic, Groningen). Abbreviations: EAC: Ethylaminocarbazole, PBS: Phosphate-Buffered Saline.*

Step	Solution	Description	Time
Blocking	2 % skim milk-powder	Orbital shaking	30 min
Rinse	Bidist water	Wash 3 times	3 × 1 min
Binding A	100 µl goat anti-human IgG-Fc in 100 ml 0.2 % skim milk-powder	Orbital shaking	30 min
Rinse	0.9 % NaCl	Orbital shaking	5 min
Binding B	100 µl rabbit anti-goat HRP-conjugated in 100 ml 0.2 % skim milk-powder	Orbital shaking	30 min
Rinse	Bidist water	Wash 3 times	3 × 1 min
Staining	Dissolve 1 EAC-tablet (20 mg) in 20 ml methanol (1 h in the dark and covered). After dissolving: Add to 100 ml 0.2 M Na-Acetate pH 5.1 Add 100 µl H_2O_2 (30 %)	Shake well	20 min
Rinse	Bidist water	Wash 3 times	3 × 1 min
Drying	Dry with a hair blower	Not too hot!	5 min

Selective immuno reactions by use of antigen-precoated membranes:
(Courtesy of AJE Green, National CJD Surveillance Centre Edinburgh)
The antigen is reconstituted with 1 ml H_2O_{Bidist} 15 min without shaking. After 15 min vortexing the Antigen is diluted in 30 ml PBS. Incubate the membrane overnight while shaking. Block the membrane afterwards with 2 % Marvel in PBS for 1 hour. Rinse with water and place in 0.2 % skim milk powder as required. Figure 18.

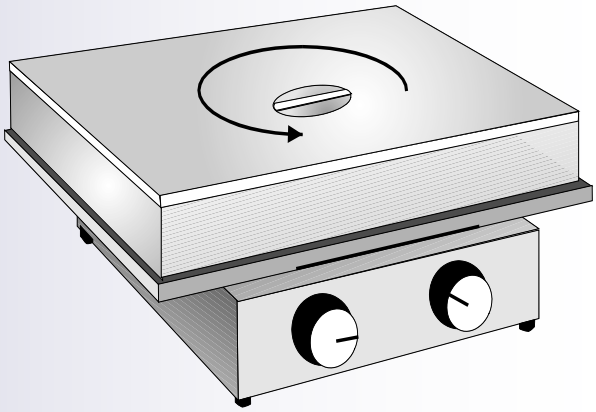
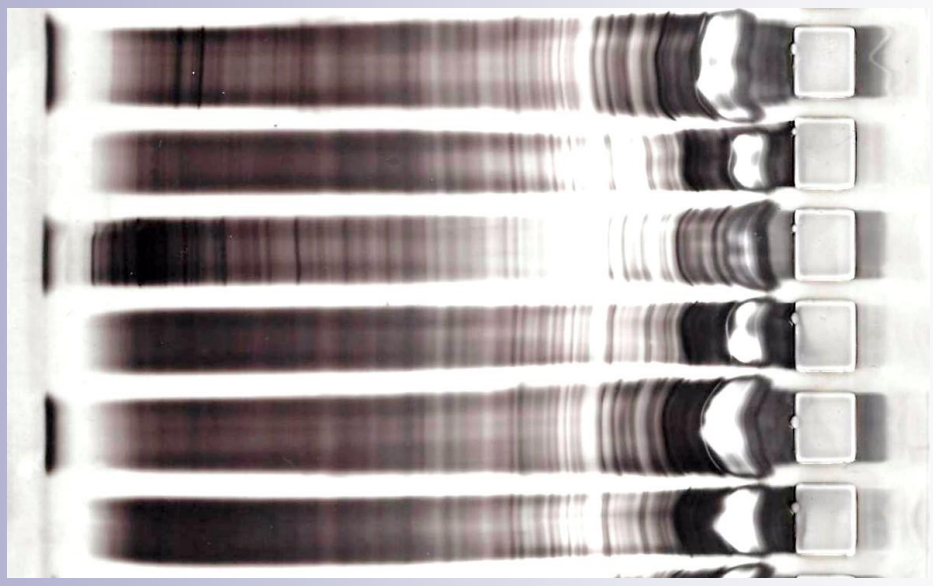


Fig. 18: Visualization procedure on a horizontal shaker

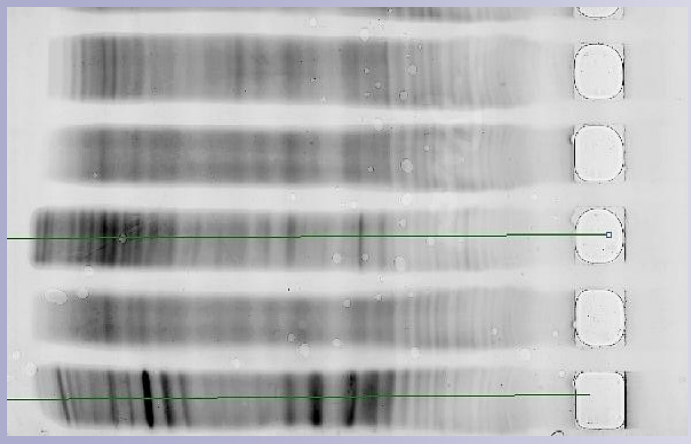
A Result after Ammoniacal Silver-Staining

After the general ammoniacal silver-staining the gel should look like this:

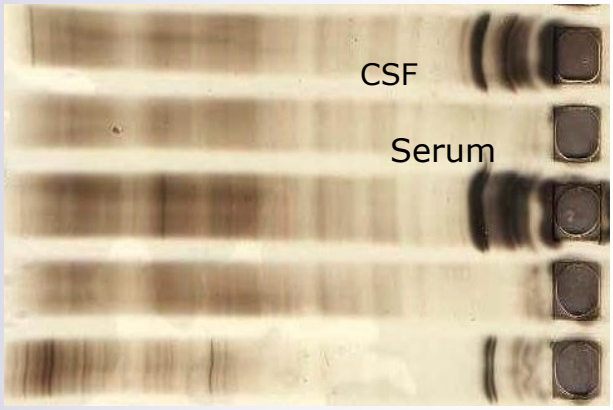


B Result after Immuno-Fluorescence and Immuno-Fixation

The immuno-fluorescence method requires an fluorescence imager but no further staining.
After the immuno-fixation and the following silver-staining the gel should look like this:
The bands next to the slots of the CSF-lanes are the Transferrin-bands.



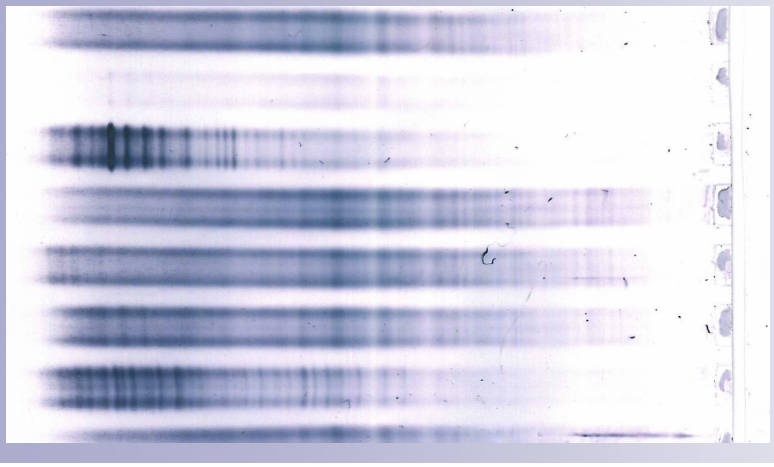
Immuno-Fluorescence visualized by a fluorescence imager



Immuno-Fixation followed by Silver staining. Courtesy of G.Cox, Morriston Hospital, Swansea

C Result after Immuno-Staining

After blotting and immuno visualization the result should look like this:



Courtesy of D.Zeman, CSF-labor, Laboratory Diagnostics, University Hospital Ostrava

Interpretation of Results

General: The bands in the IgG-region produced by the cerebrospinal fluid (CSF) and the bands expressed in the serum (S) from the same patient are compared to each other. Please consider that some proteins are specific for the CSF (Cystatin C , ß-Trace and Transferrin).

Scheme of Clinical Findings:

(according to the DGLN (Deutsche Gesellschaft für Liquordiagnostik und Klinische Neurochemie e.V.)

type **I:** CSF and serum show no or only 1 additional band: Negative, no intrathecal synth.

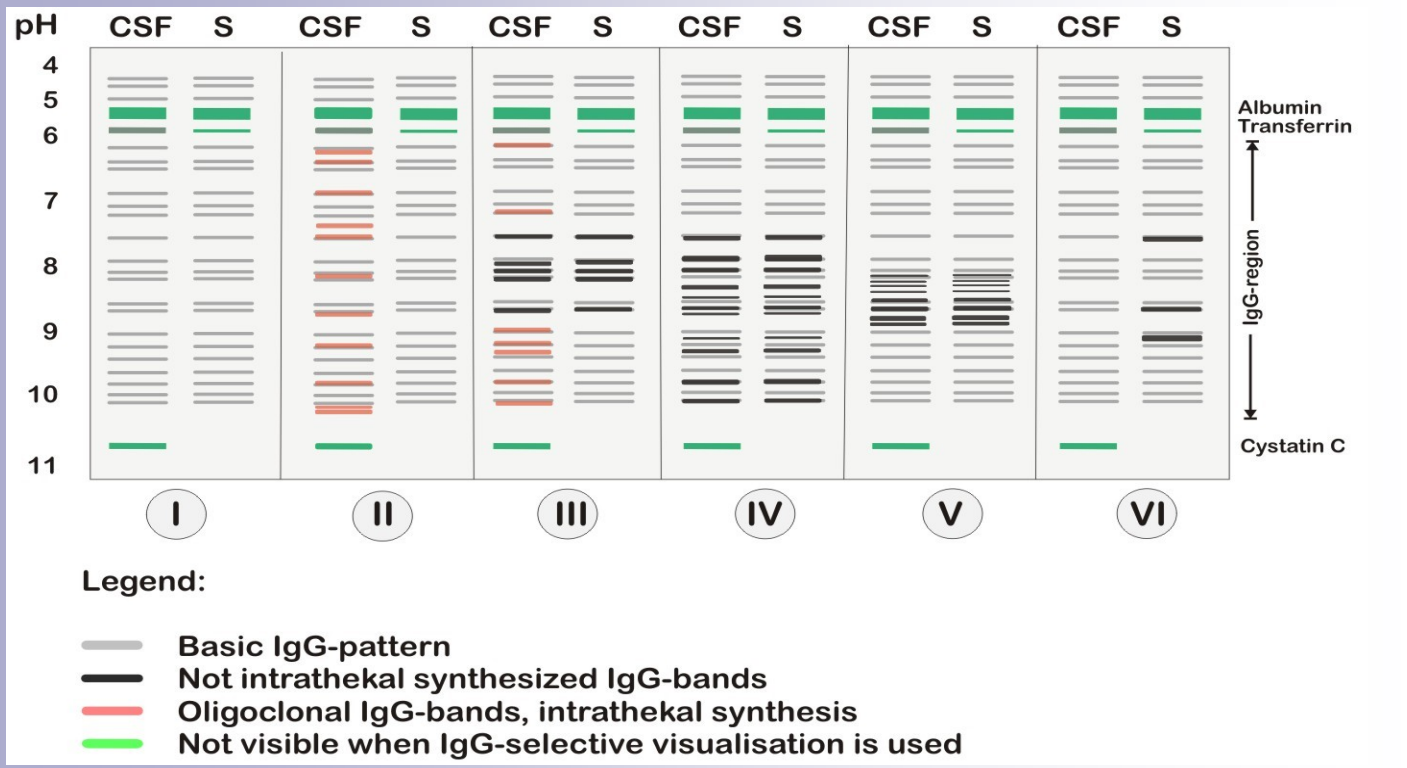
type **II:** CSF shows 2-3 or more (IgG-spec. stain, general stain: 4 or more) supplementary oligoclonal bands (not belonging to the basic pattern): Positive, intrathecal synthesis.

type **III:** CSF shows 2-3 or more (IgG-spec. stain, general stain: 4 or more) supplementary oligoclonal bands in addition to identical bands in both lanes: Positive, intrathecal synthesis.

type **IV**: Identical bands (not belonging to the basic pattern) in serum and CSF: Negative, no identical synthesis, systemic desease.

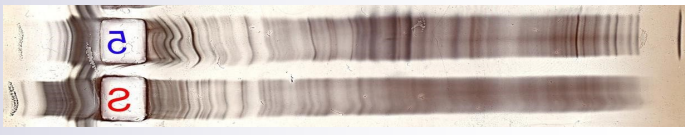
type **V:** CSF and serum show significant bands. Systemic desease, para-proteins. Negative, no intrathecal synthesis.

type **VI**: Serum shows supplementary oligoclonal bands. Negative, no intrathecal synthesis. See also: *http://www.uke.uni-hamburg.de/extern/dgln/instand.htm*

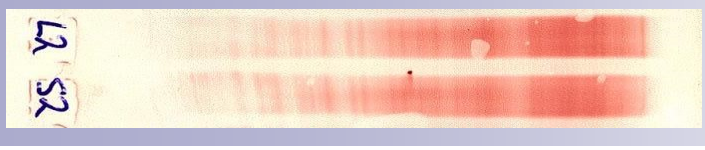


Examples: *(black bands=general silver stain, red colour=IgG-selective stain)*

type 1: negative



type 2: positive

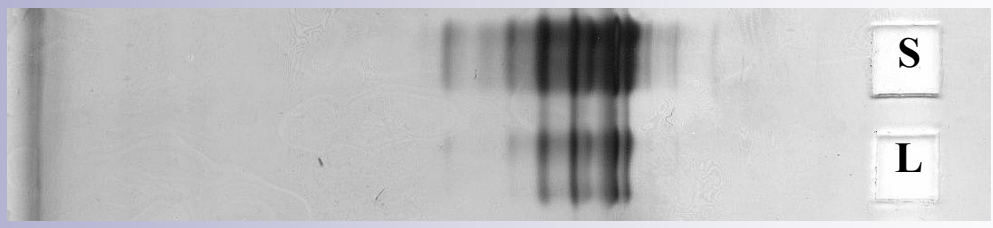


Trouble Shooting - General

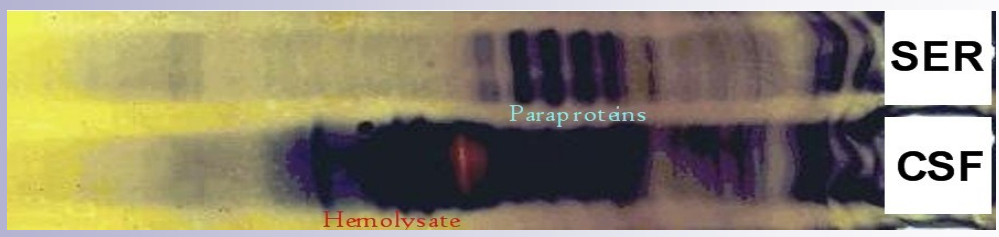
Symptom	Cause	Remedy
Voltage applied, but no current. Samples and their colour remain in the slots.	No internal connection in the chamber. *or:* Electrodes have no contact with gel surface.	Check internal cables in the chamber. Lower the electrodes onto the gel surface. Follow the manual carefully.
Lanes have different widths. Sera and CSF stain differently.	Sera and CSF have been diluted wrongly.	Use the Sample-Diluter for all sample-dilutions. Do not forget the pre-dilution of the sera.
Lanes are not running straight	Not all sample slots filled	Pipet at least 25 µl Sample-Diluter. Do not let any slot empty.
At the cathode the gel is burning.	Salt load for the gel is too high. *or:* Electrode solutions and strips were used.	Do not apply solely CSF-samples without sera in between. Do not use PBS for dilution! Do not apply electrode strips.
Silver staining does not function at all	Inadequate reagent quality	Use the chemicals as listed on page 2
Silver staining has (nearly) no contrast. Check #1.	Formaldehyde-containing liquids or the citric acid solu-tion older than 1 day.	Check reagents and water quality. Clean the tubings of the Autostainer!
Silver staining has (nearly) no contrast. Check #2.	Water has not sufficient qualor the silver-staining.	Perform the „Droplet-Test" for checking the water-quality: http://www.electrophoresis-development-consulting.de/html/droplettest.html **Do not use plastic ware!!!**

Trouble Shooting - Interpretation

Using general visualization also non-IgG proteins will show up in the IgG-region, which could be mis-interpreted as oligoclonal bands. An IgG-specific visualization (e.g. immuno-fixation) will prevent this! See picture below: The additional bands in the CSF are not IgG but Hemolysate bands. („L"=CSF)



General Silver-Staining: Hemolysate proteins show up in the IgG-region and can be mis-interpreted.



Immuno-Fixation: Hemolysate proteins are not displayed, only IgGs are visualized.