

ISOELECTRIC FOCUSING OF SERUM AND CEREBROSPINAL FLUID ON *FocusGels 6-11 24S*



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In native and in SDS-electrophoresis the Immunoglobulin G fraction (IgG) shows only 1 slightly broadened uniform band. Different oligoclonal IgG-fractions can only be separated from each other when the isoelectric focusing (IEF) is chosen as separation technique.

The cerebrospinal fluid (CSF) is an immuno-privileged space. Normally only a few T-Lymphocytes migrate through the blood-brain barrier, fig. 1. In the case of a disease the amount of Lymphocytes increases in the CSF. So both, the serum and CSF per patient has to be determined to see if there are additional oligoclonal bands. Then special CSF-based metabolic processes are going on.

3 visualization procedures can be done: 1. General Silver Staining: A special ammoniacal silver-staining (fig.1) stains all proteins 2. Immunostaining after a contact-blot (fig.2): Only IgGs will be visualized. 3. Immunofixation followed by a silver staining will also only stain only IgG bands within the gel (fig.3).

FocusGel 6-11 24S are special gels with slots for each 25µl sample volume. The pH-gradient is optimized and spread in the region between 6 and 11.

Result

FocusGels are washed, dried and rehydrated gels, ready to use for the isoelectric focusing, see figure 2. These gels are not toxic! The ampholine-cocktail forming the pH-gradient in the electric field was never in contact with the polymerization chemicals: No destroying and no aging of the ampholines occurs as low resolution and thick brown stripes after the silver-staining, see figure 3.

After performing a contact-blot an immuno-staining can be performed, see figure 4.

Interpretation

CSF („Liq“ with the arrows) shows additional oligoclonal IgG-bands compared to the serum („Ser“ above). *Positive, type 2! (Intrathekal IgG-synthesis).*
CSF („Liq“ without arrows) shows no additional bands compared to the serum („Ser“ above). *Negative, type 1!*

(No picture) Monoclonal bands in the cerebrospinal fluid, but no additional bands compared to the corresponding serum (*Systemic para-proteins, type 5, oligoclonal bands=type 4!*).

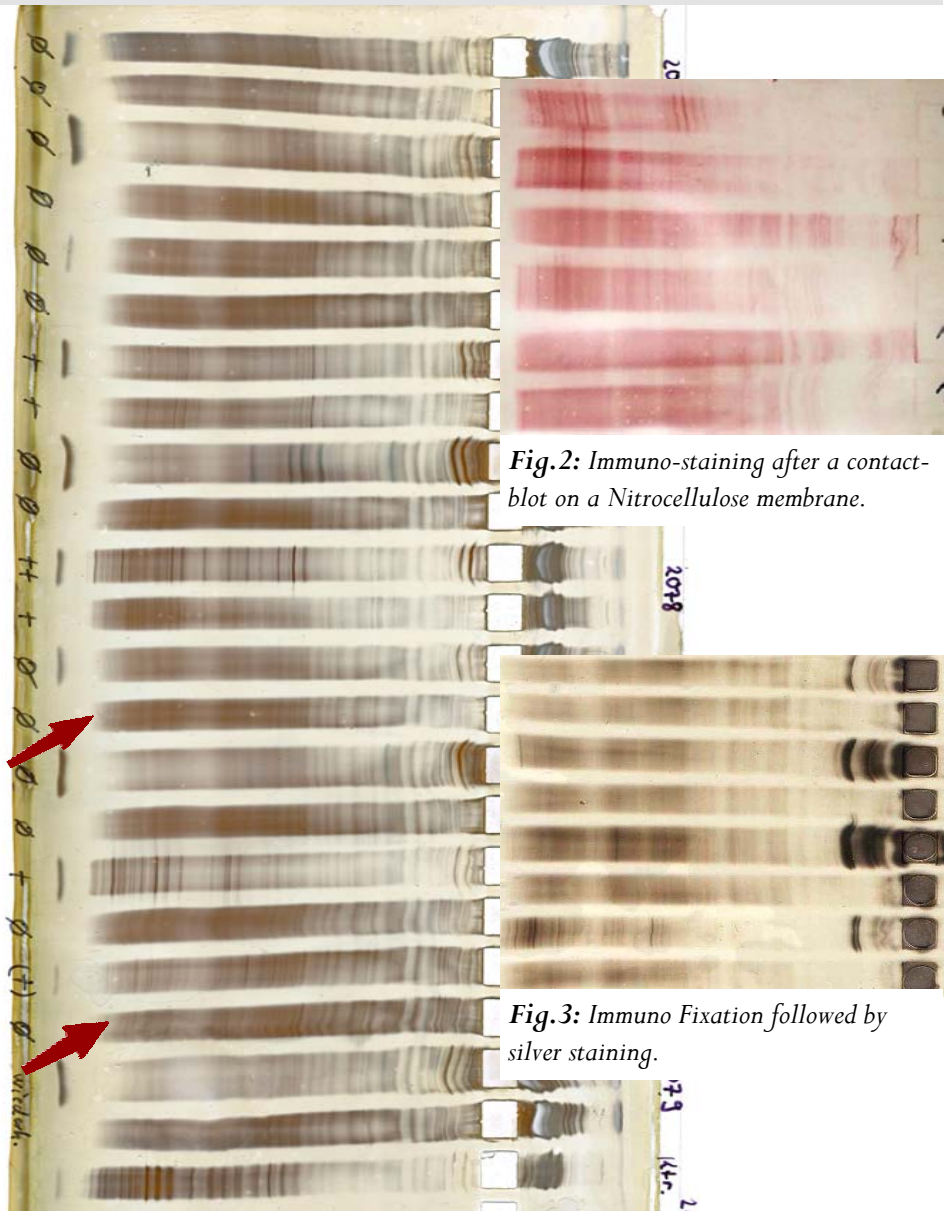


Fig.2: Immuno-staining after a contact-blot on a Nitrocellulose membrane.

Fig.3: Immuno Fixation followed by silver staining.

Fig.1: FocusGel 6-11 24 S: IEF of sera and CSF. 6 patients are applied, serum and CSF (above the sera) one beside each other. Ammoniacal Silver-staining.

Consumables

FocusGels 6-11 24S, 5 gels for IEF of CSF and serum, 24 lanes à 25µl

ETC 1006-03

Sample Diluter IEF, 100 ml (contains ampholytes, detergents, dyes)

ETC 1004-08

Nitrocellulose blotting-membrane 0.45 µm. Silver-staining and Immuno-staining chemicals see next page.

1. Silver-Staining

Table 1: General silver-staining

step	reagent	volume	temp	time
1. Fixing	20% trichloroacetic acid (w/v)	200 ml	20 °C	45 min
2. Washing	H ₂ O dist	200 ml	20 °C	5 min
3. Rinsing I	50 % methanol / 10% acetic acid (v/v)	200 ml	20 °C	40 min
4. Rinsing II	5% methanol / 7% acetic acid	200 ml	20 °C	20 min
5. Incubation	2.5 % glutardialdehyde / 1.75 % sodium-acetate	200 ml	20 °C	15 min
6. - 9. Washing	H ₂ O dist (optional: Hold overnight in the last step)	3 x 200 ml	20 °C	20 + 15 + 10 + 10 min
10. Silvering (Prepare freshly before use!)	<u>Solution 1:</u> Dissolve 250 mg AgNO ₃ in 1 ml H ₂ O dist. <u>Solution 2:</u> 40 ml H ₂ O 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 ₂ O (Or see the Silver-Staining Kit manual)	200 ml	20 °C	40 min
11.-12. Washing	H ₂ O dist (cold!)	2 x 200 ml	20 °C	1 + 5 min
13. Developing (Prepare freshly before use!)	0.0025% citric acid + 100 µl formaldehyde in 200 ml with H ₂ O dist (Or see the Silver-Staining Kit manual)	200 ml observe to stop (Set Beep!)	20 °C	5 min
14-16. Stopping & Preserving	10% ethanol, 1% acetic acid, 5% glycerol	3 x 200 ml	20 °C	10 + 10 + 10 min

2. Contact-Blot and Immuno-staining

Blot: A dry Nitrocellulose membrane is layed on the surface of the FocusGel followed by a stack of blotting papers, a glas-plate and a weight, see figure 3, 4 and 5. After 30 min the contact-blot is ready. **Visualization:** See table 2.

Table 2: The Immuno-staining

Step	Solution	Description	Time
Blocking	2 % skim milk-powder	Shake horizontally	30 min
Rinse	Bidest water	Flush 3 times	3 x 1 min
Binding A	100 µl goat anti-human IgG-Fc in 100 ml 0.2 % skim milk-powder	Shake horizontally	30 min
Rinse	Bidest water	Shake horizontally	5 min
Binding B	100 µl rabbit anti-goat HRP-conjugated in 100 ml 0.2 % skim milk-powder	Shake horizontally	30 min
Rinse	Bidest water	Flush 3 times	3 x 1 min
Staining	Dissolve 1 EAC-tablet in 20 ml methanol (in the dark and covered). After dissolving: Add to 100 ml 0.2 M Na-Acetate pH 5.1 Pipet to this: 100 µl H ₂ O ₂ (30 %)	Shake well	20 min
Rinse	Bidest water	Flush 3 times	3 x 1 min
Drying	Dry with a hair blower	Not too hot!	5 min



Fig.3: 30 min contact-blot

3. Immunofixation followed by Silver Staining from step #5

step	reagent	volume	Temp	time
Immunofixation	250 µl Rabbit Anti human IGG + 100 µl Rabbit Anti-human Transferrin in 30 ml 3 % PEG* 6000 in PBS*	30 ml	20 °C	3 h
Washing	3 % PEG* 6000 in PBS*	1000 ml	20 °C	overnight

Abbreviations: PEG=Polyethylen glycol, PBS=Phosphate Buffered Saline