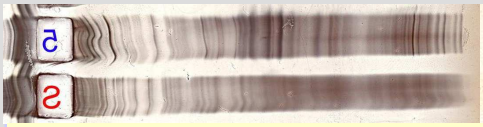
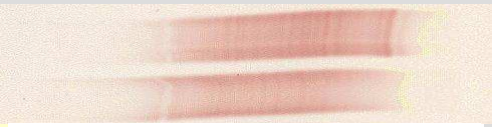
Oligoclonal IgG-pattern in IEF of CSF and Serum Samples
A comparison between Agarose- and PAG-Gels

07/13



PAG: General Silver Staining

PAG: Blot and Immunostain



Agarose: Blot and Immunostain

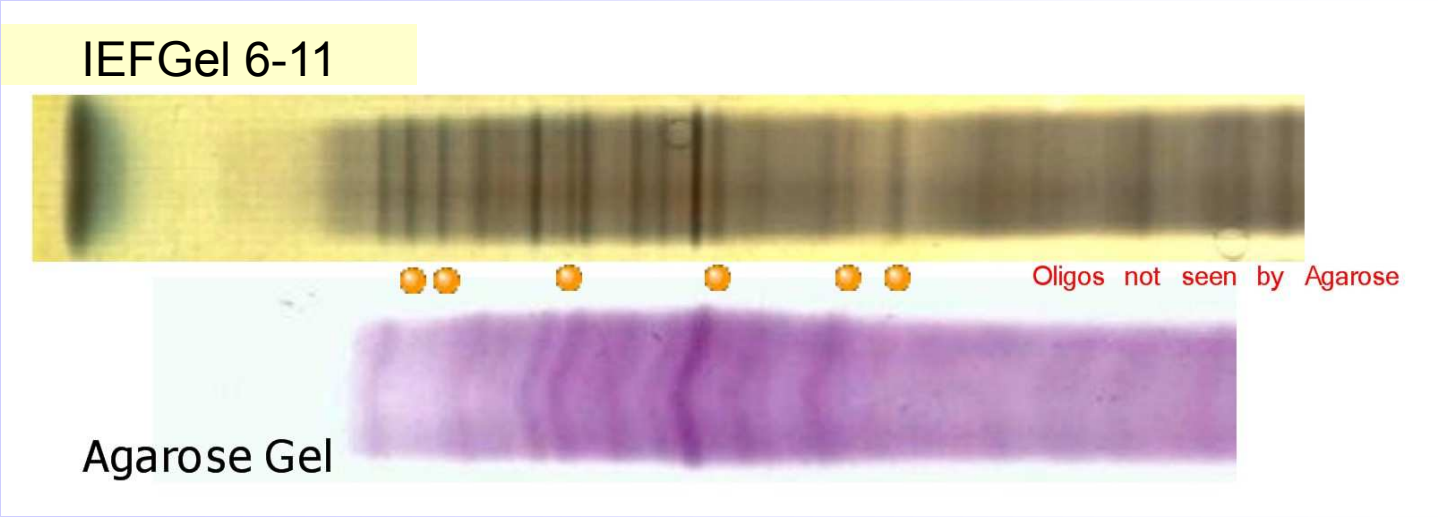
General

Isoelectric focusing (IEF) is the only method to separate different IgG molecules from each other. In all other methods, such as SDS- and native electrophoresis, the whole IgG spectrum appears as 1 broad band.

The IEF-run

Due to its high molecular weight (160 kD) oligoclonal IgG is not easy to focus in sharp bands. Normally a pH-gradient of 3-10 should be run more than 4000Vh.
Agarose gels cannot survive this procedure.
Because of intrinsic fixed negative charges in agarose gels - caused by carboxylic and sulfonic residues - there is an electroendosmotic water transport towards the cathode. This part of the gel becomes more and more wet and the anodal part will become thinner and thinner….
After about 1500 Vh the IEF with Agarose gel must be stopped!
This leads to blurred IgG bands without sufficient sharpness.
Polyacrylamide-based gels (PAG-gels) can be run up to 4500 Vh. This is enough for displaying even the finest IgG-bands.

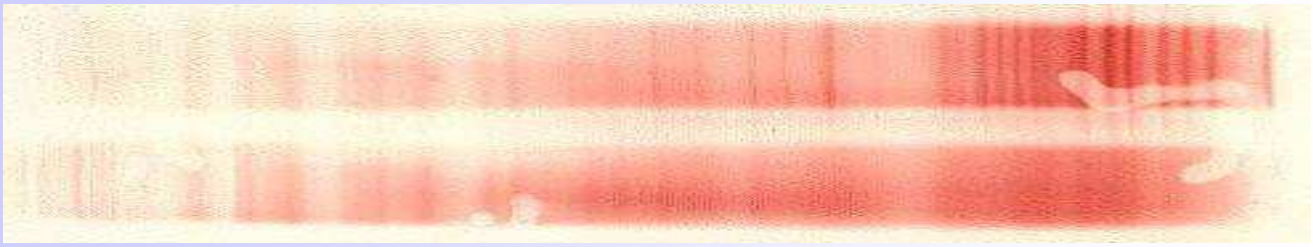
Please compare identical CSF-samples on Polyacrylamide- and Agarose gels below.



Comparison: PAG and Agarose Gel

For specific information of all procedures described here please follow our manual „IEFGel 6-11" on EDC's website, rubrique „Download".

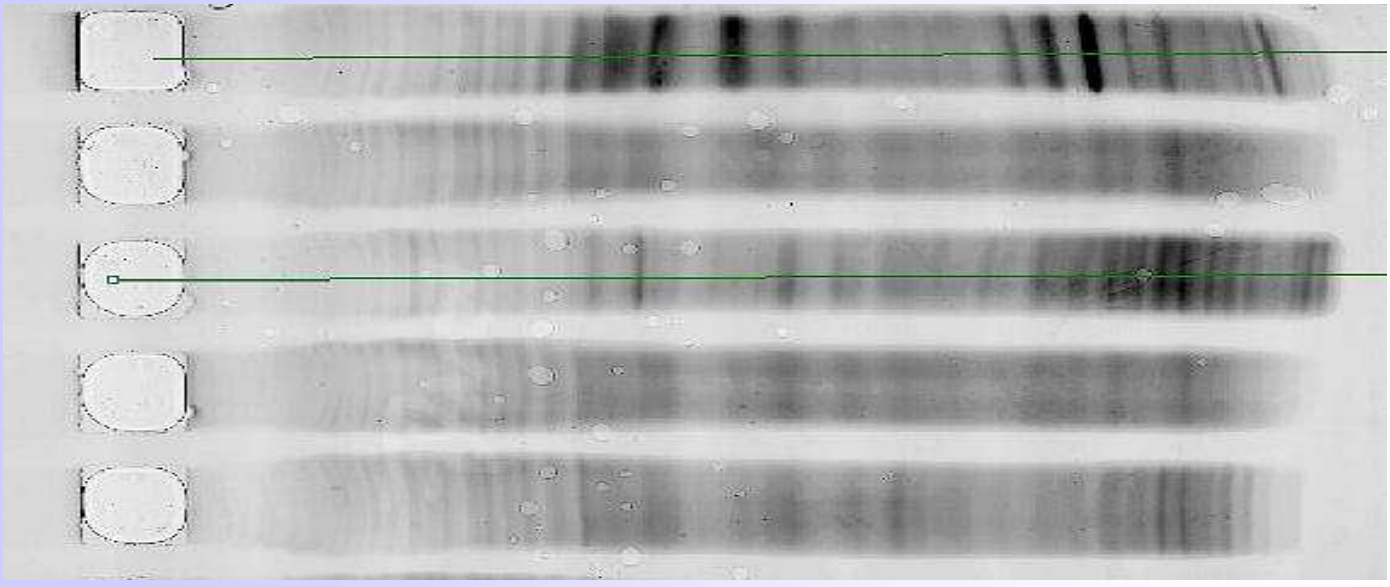
Blotting with Immunostain: IgG-selective visualization is a little less sensitive than silver stain. 20 ng IgG of a healthy person gives nearly no background.
An additional procedure, the contact blotting, has to be performed.



Selective Immunoreactions by use of Antigen-precoated membranes: The specificity of IgGs can be typed by coating the blot membrane with antigens —-> No picture.

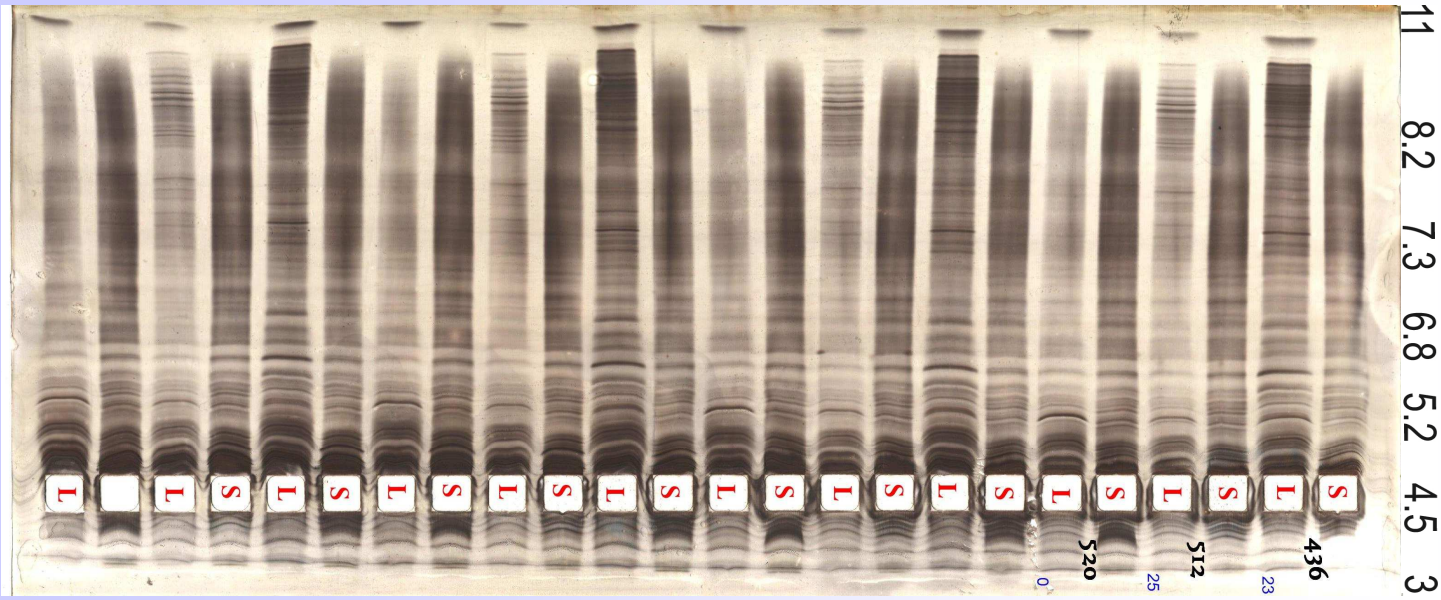
Immunofixation with Silver Staining: IgG-selective staining, very sensitive, only 5 ng of IgG is enough. Needs an overnight step (elution of the excess antibody). This is the only procedure that combines the selectivity of immuno based visualization and the sensitivity of a silver-stain. Not possible on Agarose gels!

Immuno-Fluorescence: IgG-selective staining, sensitive, quick and easy!
After reaction with a T-Rex-chromogen conjugated antibody (Dyeagnostics, Halle, Germany) and the washing-out of the exceed antibody, the gel goes directly to the fluorescence-imager. Not possible on Agarose gels!

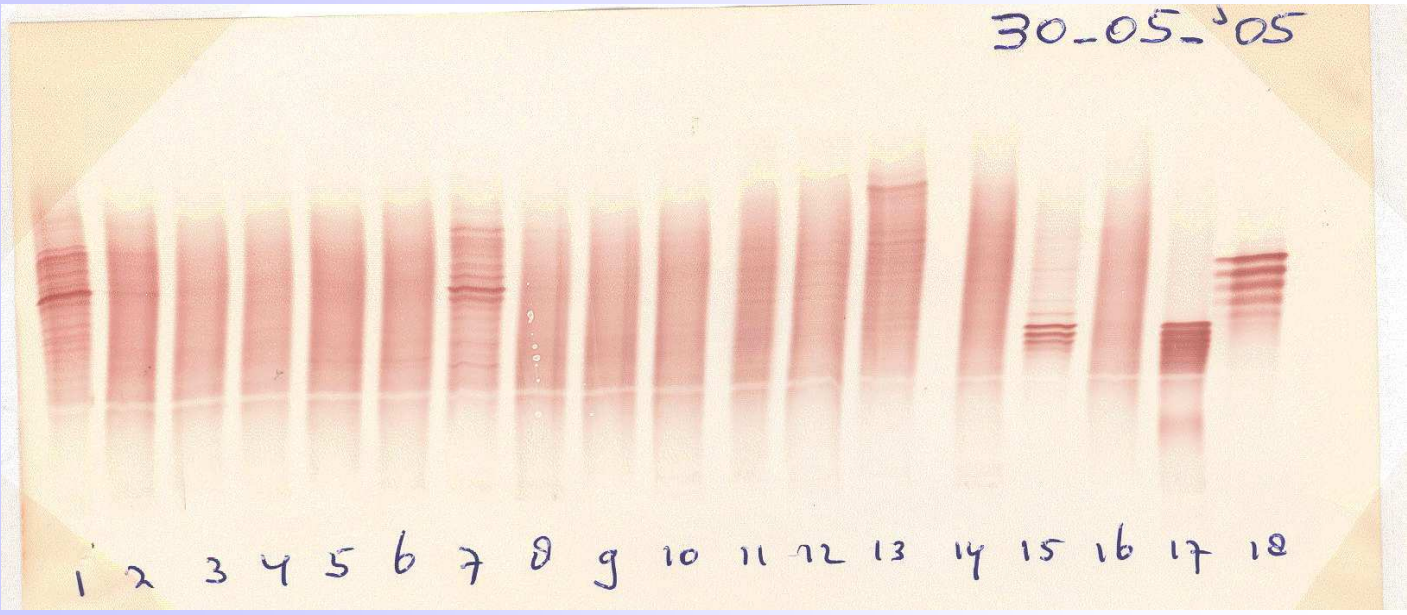


The comparison of the different methods: IEFGel 6-11 versus Agarose gels
EDC has developed a special gel to overcome all problems in oligoclonal IgG typing: EDC produces non toxic PAG gels with a pH gradient optimized in the important region of pH 6-11. For easy sample application these gels have 2 slots for every patient.
All 4 important visualizations can be performed with IEFGel 6-11 24S, 40S or 80S.

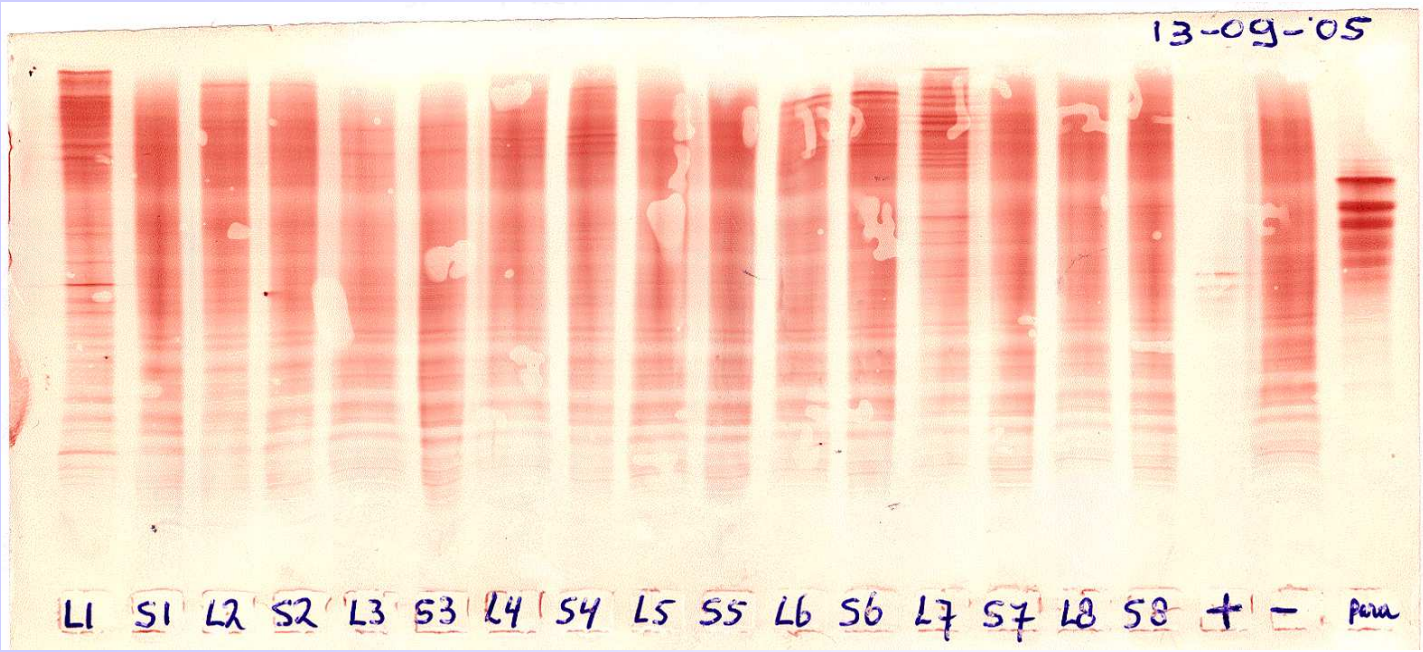
IEFGel 6-11 24S with silver staining (not possible on Agarose gels)
Fine pattern, sensitive staining. Cystatin C is visualized! The optimized pH-gradient stated on the right side of the picture. 20 ng in 22 µl was applied per lane.



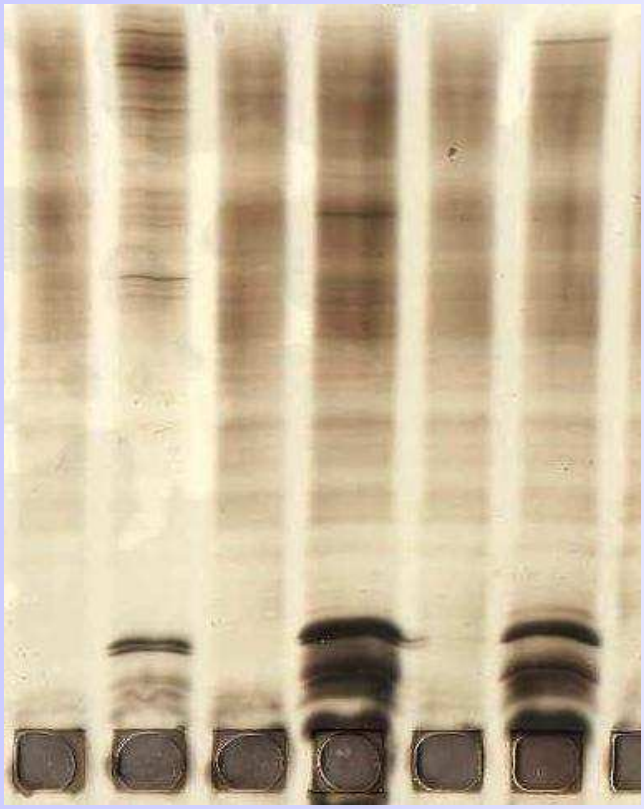
Agarose gel, blotted and stained with immuno staining
Faint background, no background pattern e.g. in lane 3 and 4. 20 ng IgG was applied.

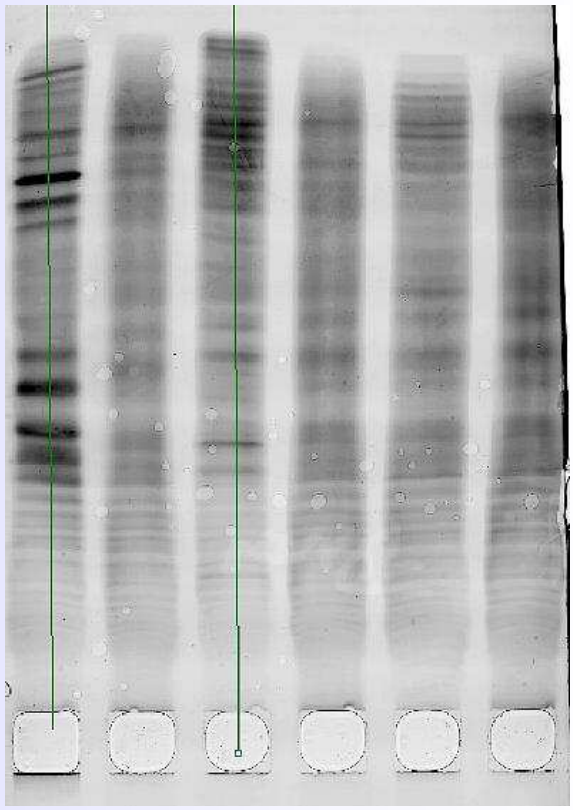


IEFGel 6-11 24S, blotted and stained with immuno staining
Please note: The IEF maintaines the band sharpness until the end of the visualization procedure. The background pattern is visible e.g. in L2 and S2.
20 ng IgG was used.



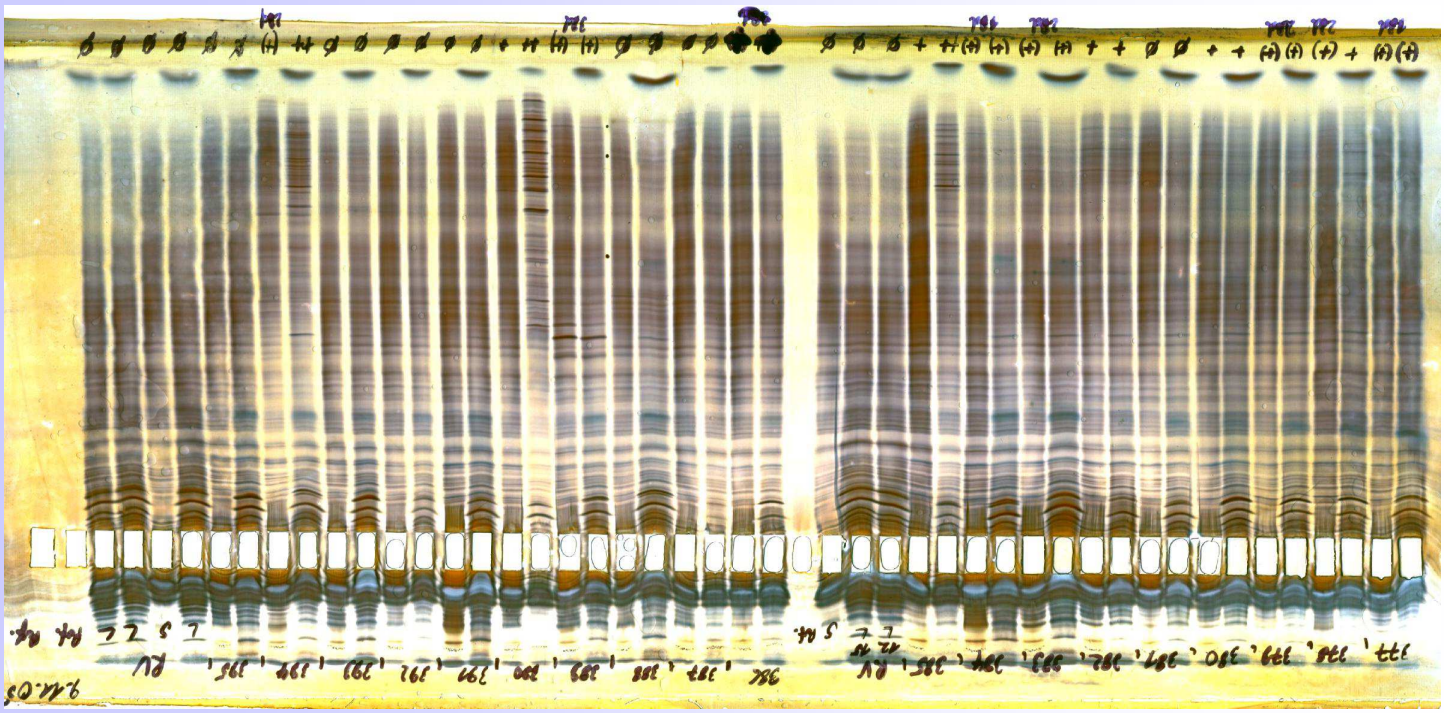
IEFGel 6-11 24S, Immunofixation and Immuno-Fluorescence
Left picture below shows Immuno-fixation followed by silver-staining: A small amount of Anti-human transferrin is added to distinguish cerebrospinal fluids from the sera.
Only 5 ng of IgG is used per lane.
Right picture below shows the result of a fluorescence-imager.
15 ng IgG was applied.
Both methods are not possible on Agarose gels.
This procedur fits into a 8 hours working-day.



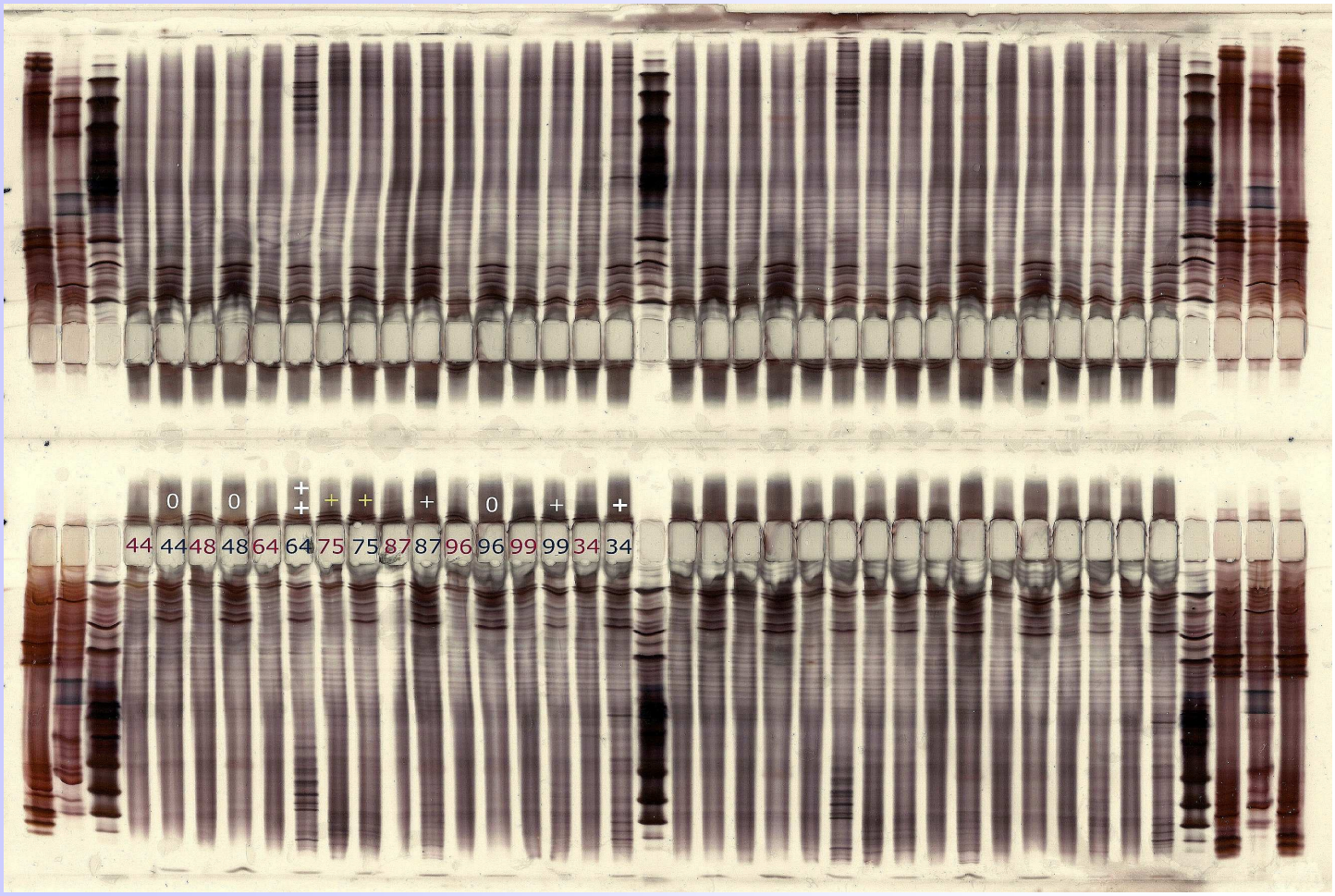


IEFGel 6-11 40S

For laboratories with high sample throughput EDC offers the IEFGel 6-11 with 40 slots for 20 patients, res. 80 slots for 40 patients on 1 gel.
See pictures below.



IEFGel 6-11 40S(lots): 10 ng IgG was applied in 12 µl



bidirect IEF on IEFGel 6-11 80S(lots): 10 ng IgG was applied in 12 µl