

PRACTICABILITY OF SIMULTANEOUS Gc AND Tf SUBTYPING IN BLOOD STAINS

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Gc and Tf subtyping techniques of blood stains by isoelectric focusing (IEF) has been developed in the past years (for example BERGHAUS and STAAK, 1982). Since there is considerable variation in gene frequencies IEF for simultaneous Gc and Tf subtyping of blood stains may be a useful tool that dramatically increases the discriminating capabilities of these polymorphisms. We therefore developed techniques (ampholyte gel, 20 cm separation distance; Immobiline^R gel, 10 cm separation distance) to analyse blood stains in one focusing.

Materials and methods

Serum specimens for subtyping had been pretreated by mixing 1 drop serum with 3 drops of 0,5 g% ferrous ammonium sulfate. Blood stains of known subtypes were made on glass plates allowed to dry and stored at room temperature. Prior to focusing blood stains were weighted and diluted in 0,5 g% ferrous ammonium sulfate. All samples had been stored overnight at 4° C.

The technique of IEF using carrier ampholyte on 0,1 mm polyacrylamide gels (RADOLA, 1980) has been modified as shown in Fig. 1. The technique of IEF with immobilized pH gradients (GÖRG et al., 1983) has been modified as shown in Fig. 2.

Results

The results of IEF with carrier ampholytes for the simultaneous analysis of Tf and Gc subtypes is shown in Fig. 3: In the upper part the Gc subtypes after immunofixation with monospecific anti-Gc antiserum and in the lower part

Fig. 1: IEF-technique with carrier ampholytes for the simultaneous analysis of Gc and Tf subtypes.

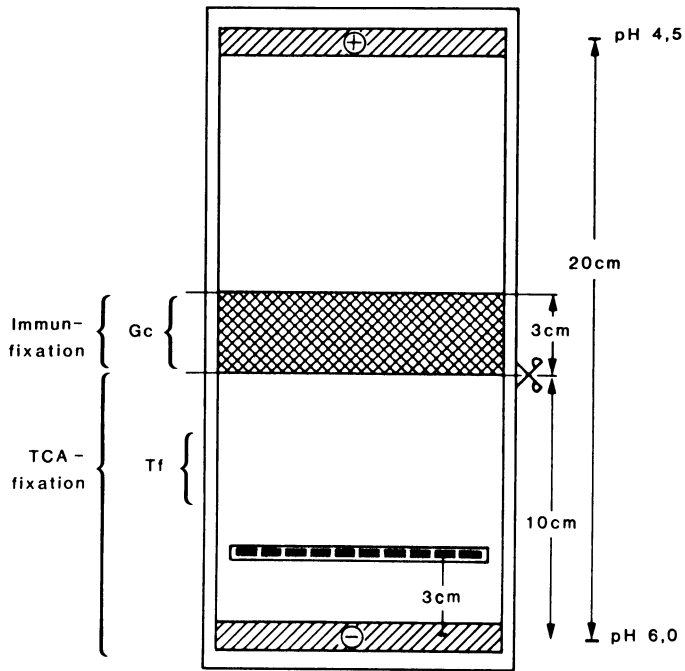
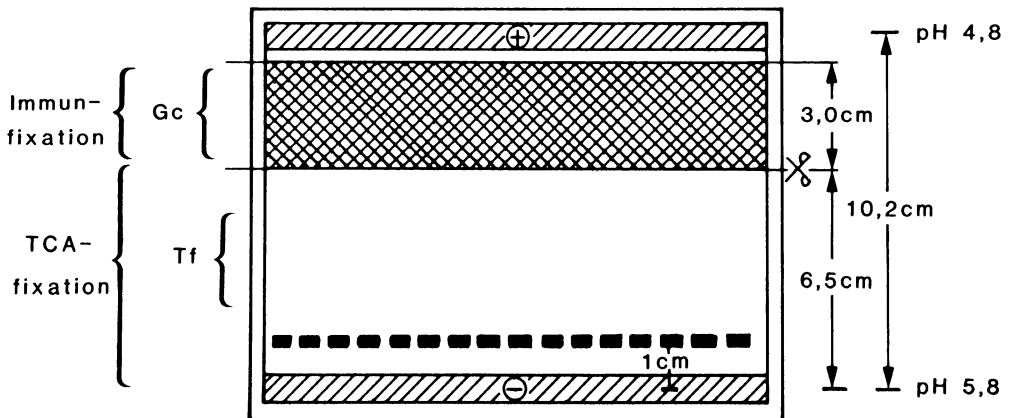


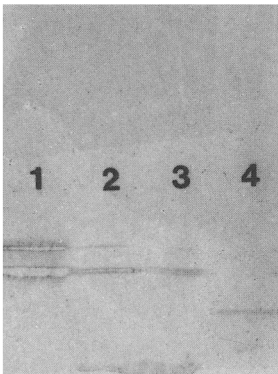
Fig. 2: IEF-technique with immobilized pH gradient for the simultaneous analysis of Gc and Tf subtypes.



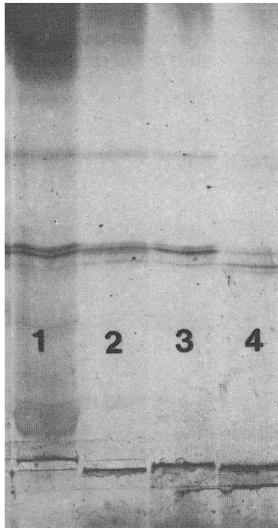
the Tf subtypes in the same polyacrylamide gel after TCA-fixation. Sample volume: 10 μ l of a solution of 10 mg blood stain + 50 μ l ferrous ammonium sulfate.

Fig. 4 is an illustration of the results of the IEF with immobilized pH gradient pH 4,8 to pH 5,8: In analogy to Fig. 3 in the upper part the Gc-phenotypes and in the lower part the Tf-phenotypes. Sample volume: 20 μ l of a solution of 10 mg blood stain + 50 μ l ferrous ammonium sulfate.

Fig. 3: Subtypes of Tf and Gc in blood stains, IEF with carrier ampholytes.

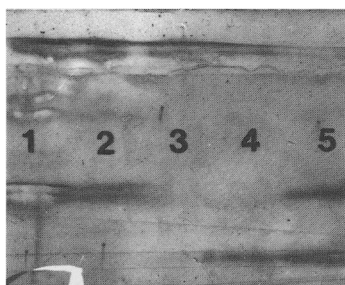


Upper part of the polyacrylamide gel: Gc-subtypes after immunofixation with monospecific anti-Gc antiserum. From left to right: Gc 1F1S reference, Gc 1F1F blood stain, Gc 1F1S blood stain, Gc 2 blood stain.

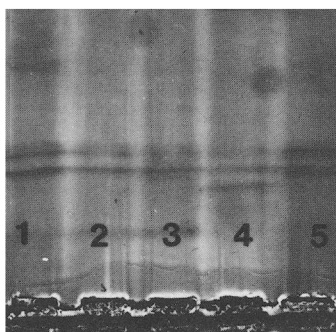


Lower part of the same polyacrylamide gel: Tf-subtypes after TCA-fixation. From left to right: Tf C1 reference, Tf C3-1 blood stain, Tf C1 blood stain, Tf C2-1 blood stain.

Fig. 4: Subtypes of Tf and Gc in blood stains by simultaneous IEF with immobilized pH gradient.



Upper part of the polyacrylamide gel: From left to right: Gc 1F1S reference, Gc 1F1S blood stain, no pattern blood stain, Gc 2 blood stain, Gc 2-1F reference.



Lower part of the same polyacrylamide gel. From left to right: Tf C1 reference, Tf C1 blood stain, Tf C3-1 blood stain, Tf C2-1 blood stain, Tf C3-1 reference.

Discussion

For the simultaneous demonstration of the genetically determined variability of the Tf and Gc system in blood stains, IEF with carrier ampholytes and IEF with an immobilized gradient were found to be suitable. IEF using carrier ampholytes shows a lesser degree of resolution than IEF with immobilized gradient.

References

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