4.3. Typing of serum monoclonal proteins

4.3.1. Immunofixation electrophoresis

For sIFE, a patient’s serum is applied to several lanes of an agarose gel, and after electrophoresis, specific antisera are overlaid on individual lanes of the gel. These antisera are typically against IgG, IgA, IgM, κ and λ, although other specificities may be useful for identifying unusual bands (e.g. IgD, IgE or fibrinogen). A lane fixed with acid (which fixes all proteins) is also included for comparison. Following removal of the antisera, gels are washed and stained with Coomassie Brilliant Blue or Amido Black (Figure 4.3).

IFE is approximately 10-fold more sensitive than SPE, and is included in MM guidelines in the definition of a stringent complete response (Chapter 25) [1]. It should be noted that judging whether there is or is not a discrete (monoclonal) protein band present after IFE can be subjective, and this may lead to discordance in the response category assigned by different operators (Section 18.4.3) [2].

4.3.2. Immunosubtraction

Figure 4.3. Serum immunofixation

Figure 4.4. IgG immunosubtraction
Immunosubtraction can be used in place of IFE for typing the majority of monoclonal protein bands, but it is less sensitive [4]. In this technique, antibodies against IgG, IgA, IgM, κ or λ (bound to solid phase beads) are incubated with serum aliquots, then CZE is performed to determine which reagent(s) remove an electrophoretic abnormality (Figure 4.4).

This procedure works well with samples producing discrete monoclonal protein spikes and is easier to perform than sIFE [5]. However, IFE may still be required as a complementary method to determine the monoclonal protein type in a number of situations [5][6][7]. These include detection/typing of: 1) monoclonal sFLCs of the same type as the characterised monoclonal intact immunoglobulin; 2) multiple monoclonal proteins, e.g. biclonal gammopathies or oligoclonal banding; 3) “hidden” monoclonal proteins, e.g. small (<3 g/L) IgA or IgM monoclonal proteins that co-migrate with other serum proteins in the β-region; and 4) IgD and IgE monoclonal proteins.
(A) Normal serum. (B) Monoclonal IgGκ intact immunoglobulin. (C,D) Monoclonal IgDκ intact immunoglobulin with λ FLCs. Fλ: anti-free λ antisera. (Courtesy of Me Musset Hôpital Pitié-Salpêtrière – Paris, France).

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**Figure 4.4. IgGκ immunosubtraction example.**

The monoclonal protein peak is removed with addition of anti-IgG and -κ antibodies. (This figure was originally published in [3], reproduced with permission from Taylor & Francis Group).

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References


