Quantification of antigens and antibodies by immuno-electrophoresis

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Immuno-electrophoresis (IEP) separates and visualizes antigen-antibody binding by electrophoresing samples through an agarose gel. Antigens and antibodies migrate based on charge, forming visible precipitin lines at optimal ratios. Precipitin patterns allow identification and quantification of antigen-antibody interactions.

This study demonstrates the use of IEP to identify and quantify unknown antibodies through a reaction with known reference antigens of differing molecular weights and charges. Specifically, human serum albumin (HSA), ox serum albumin (OSA), and ovalbumin (OVA) were utilized given their established yet distinct migration patterns in IEP gels. The detailed IEP protocol provided includes critical steps like slide preparation, antigen loading, electrophoresis parameters, antibody incubation, washing, precipitation enhancement by freezing, and staining for visualization. Comparison of resulting precipitin line patterns and migration distances enabled the deduction that the unknown antibody solution contained anti-HSA and anti-OVA antibodies. Further calculation of antigen-antibody ratios from molecular weight values and measured precipitate quantities yielded ratios of 4:63 for the HSA interaction and 1:86 for OVA. The ratios observed in the study suggest the zone of antigen-antibody equivalence, where immune complexes are most likely to form and precipitate out of solution. While immunoelectrophoresis (IEP) has limits in precision and detection compared to newer immunologic assays, its simplicity, cost-effectiveness, and capacity for quick antigen-antibody binding screens mean it retains high utility. This research demonstrates IEP's continued value for evaluating antibody specificity and levels, identifying pathogenic antigens, titering antibodies, and helping diagnose diseases. The paper supplies helpful details on executing IEP, plus a discussion of key concepts, uses, and pitfalls to serve as a reference for those applying this fundamental immunologic method.

Appendix

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