

# Alpha, beta, gamma of serum proteins: a long history of successfully resolved problems

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Wheeler SE, Shurin MR. Alpha, beta, gamma of serum proteins: a long history of successfully resolved problems *Journal of Immunopathology* November-2017;1(1):1-7.

Characterization of blood proteins for clinical purposes was initiated in the middle of the nineteenth century and serum protein electrophoresis was first developed in the 1930s by a Swedish biochemist Arne Tiselius. Today, clinical analysis of human serum proteins by the electrophoretic method that evolved from the original fluid method of Tiselius, through paper electrophoresis, cellulose acetate, agarose, high-resolution agarose gel electrophoresis, capillary electrophoresis to total serum protein

electrophoresis with immunofixation is widely and commonly used in clinical diagnostic laboratories. Although screening for monoclonal gammopathies has evolved over the last five decades and resulted in highly specific and sensitive techniques and standardized testing algorithms, new treatment modalities for monoclonal gammopathies have recently brought new and unexpected challenges for clinical diagnostics laboratories. Therefore, the more sophisticated techniques of immunofixation, isoelectric focusing, improved gel resolution, serum free light chains, heavy/light analysis and quantification of immunoglobulins are important in the diagnostic work-up of a patient with suspected plasma cell dyscrasias.

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**Keywords:** Protein electrophoresis; Serum proteins; Monoclonal gammopathy

## SHORT COMMUNICATION

Immunopathology as a branch of biomedical science deals with the evaluation of the role of immunological processes in the development of diseases as well as in their diagnosis, prognosis, and treatment. Clinical immunopathology testing of immune-mediated diseases (such as immunodeficiency, autoimmune, allergic and infectious diseases, cancer, inflammatory disorders, transplantation-associated diseases and conditions and other disorders) is based on an array of constantly improving methods and techniques including: immunohistochemistry and immunocytochemistry, flow cytometry, antibody detection assays, immunoassays, multiplex-based assays, cytogenetic procedures, nephelometry, spectrophotometry, chromatography, mass spectrometry and other methods. Among these modern immunopathology tools, protein electrophoresis is one of the most widely used tools for detection of numerous pathological conditions and identification of proteins in biological fluids. No other technique delivers as much information about the purity and composition of a sample as rapidly and with such little effort and expense.

Characterization of blood proteins for clinical purposes was initiated in the middle of the nineteenth century and the term “globulin” was introduced in 1862 to describe proteins that were insoluble in water. By the early twentieth century, serum proteins were generally divided into two main groups - albumin and globulins, depending on the precipitation of ‘globules’ upon the addition of sodium sulfate to serum specimens. This reaction forms a white deposit – albumin (Latin *alba*, white), after eliminating the salt by dialysis and evaporating the water. The albumin/globulin ratio was used at that time for determining liver pathology.

Protein electrophoresis was first developed in the 1930s by a Swedish biochemist Arne Tiselius (Arne Wilhelm Kaurin Tiselius) using a liquid medium, a U-shaped electrophoretic cell and an optical system to detect the refraction of light by proteins as they moved through the tube [1]. In the Tiselius apparatus, the electrophoretic migration of proteins was followed at the two boundaries formed by a U-tube of protein solution with buffer above the two vertical arms. This technique was termed

moving boundary electrophoresis. Although simple by modern standards, this early method was instrumental in the original definition of the major fractions of human serum proteins, as Tiselius observed four distinct bands: the most rapidly moving was albumin, the others he named in order of decreasing mobility alpha-, beta- and gamma-globulin. Thus, were born the names for the classic groups of serum proteins. Of note, Tiselius's manuscript describing his new methodology was rejected by the *Biochemical Journal* as being “too physical”. The paper was accepted by the journal of *Transactions of the Faraday Society* and published in 1937 [2]. That journal is no longer in existence. Eleven years later, Tiselius received the Nobel Prize for his achievements in the field of protein electrophoresis that had begun with his doctoral thesis in the 1930s. It is important to mention that the alpha-, beta- and gamma- globulins were not described in the publication in *Transactions of the Faraday Society*, but they are well defined in other papers by Tiselius that the *Biochemical Journal* agreed to publish later in the same year [2,3]. Thus, we can state now that Tiselius’ description of four components in serum – albumin, alpha-, beta- and gamma globulin, earned him a Nobel Prize in Chemistry in 1948 “for his research on electrophoresis and adsorption analysis, especially for his discoveries concerning the complex nature of the serum proteins”.

The 1950s saw many successful novel electrophoretic methodologies, such as paper electrophoresis (now forgotten but it was a landmark in separation science), use of starch and sucrose as separation media, the development of polyacrylamide gels that allowed for control of pore size in a stable matrix. The development of electrophoresis on agarose gels revolutionized not only a biochemistry but also clinical chemistry. The 1960s continued improvements to electrophoretic separation technologies with isoelectric focusing and sodium dodecyl sulphate-polyacrylamide gel electrophoresis that allowed discriminating proteins based on surface charge and molecular mass [4]. Electrophoretic analysis became generally accepted as a protein analytical tool after the development of denaturing, discontinuous stacking systems in the 1960s and the availability of commercial block gel systems in the 1970s. The 1970s also brought a fully instrumented approach to electrophoresis, as well as 2D electrophoresis

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*Received: 25 September 2017, Accepted: 31 October 2017, Published: 15 November 2017*

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and silver staining techniques. It was not until the 1980s that immobilized pH gradients and capillary zone electrophoresis allowed for the fine tuning of protein separation that we expect today [4].

Today, clinical analysis of human serum proteins by the electrophoretic method that evolved from the original fluid method of Tiselius, through paper electrophoresis, cellulose acetate, agarose, high-resolution agarose gel electrophoresis, capillary electrophoresis to serum protein electrophoresis with immunofixation is widely and commonly used in clinical diagnostic laboratories. Serum protein electrophoresis with immunofixation is used to identify, characterize and monitor various disease states where abnormal serum protein levels are suspected. Although such disorders include solid tumors, acute and chronic infections, trauma, connective tissue diseases and liver diseases, the majority of clinical analysis is to identify lymphoproliferative disorders with a monoclonal gammopathy: multiple myeloma, monoclonal gammopathy of undetermined significance, Waldenström macroglobulinemia, monoclonal gammopathy of renal significance, primary amyloidosis, chronic lymphocytic leukemia, lymphoma. A characteristic feature of monoclonal gammopathies is an increased proliferation of clonal plasma cells associated with a detectable overproduction of an immunoglobulin or one of its components called monoclonal component or M-protein.

Electrophoretic distribution of protein in normal serum demonstrates an immunoglobulin enriched gamma region with a broad distribution because of production of numerous polyclonal immunoglobulins by normal plasma cells. In monoclonal gammopathy, however, a band of M-protein (monoclonal immunoglobulin(s) or its component heavy or light chain) is seen as a restricted area in the electrophoresis migration pattern. Though a distinct protein band is seldom a false-positive result, visualization of a restricted band or non-homogeneous distribution of proteins in the gamma region of electrophoresis require immunofixation to confirm and to isotype the heavy and light chains of monoclonal immunoglobulin. Once a paraprotein has been detected and a specific diagnosis has been confirmed, the quantitation of the monoclonal immunoglobulin is commonly used as a marker of the malignant plasma or B cell clone's response or resistance to therapy [5]. The analytical sensitivity of modern serum protein electrophoresis varies between 500 and 2000 mg/L and thus a major limitation of this technique is the limited ability to distinguish low-level monoclonal proteins. Although sensitivity of serum immunofixation electrophoresis is 10-15 times higher, neither of these methods can reliably detect low levels of monoclonal light chains circulating in patients with oligosecretory diseases, such as light chain multiple myeloma, AL-amyloidosis and light chain deposition disease. Development of additional tests, like nephelometric detection of serum free light chains, has begun to remedy the problem of limited sensitivity of electrophoresis. The utilization of these assays has allowed for improved diagnosis of numerous monoclonal plasma cell disorders, from the asymptomatic monoclonal gammopathy of undetermined significance and smouldering multiple myeloma to the symptomatic multiple myeloma, Waldenström's macroglobulinemia, amyloidosis and plasmacytoma [6,7].

A related diagnostic problem is associated with the common IgA M-protein migration in the beta region on serum protein electrophoresis, which results in the inability to quantify the M-protein due to masking by other serum proteins that co-migrate in this region. Recent development of another immunoassay detecting both IgA heavy/light chain levels and ratios seems to provide some help in this problem: this assay quantifies the involved/uninvolved isotype by calculating the ratio of IgAkappa/IgAlambda, which is considered indicative of clonal proliferation [8]. Furthermore, in addition to IgA, the heavy/light (Hevylite) method also permits quantitative analysis of heavy/light chain pairs of IgG and IgM immunoglobulins and has a promising ability to improve the standard analytic tools by assessing the concentration and ratio of the levels of both abnormal and physiological immunoglobulins (heavy/light chain pair suppression), which is not possible with serum protein electrophoresis or global quantitative analysis of immunoglobulin isotypes [9]. Together, serum protein electrophoresis, serum immunofixation, free light chain measurement, heavy/light quantification methods, and nephelometric measurements of total immunoglobulin in serum are a group of modern

commonly used laboratory tests required for the management of plasma cell proliferative disorders.

Although screening for monoclonal gammopathies has evolved over the last five decades and resulted in highly specific and sensitive techniques and standardized testing algorithms, new treatment modalities for monoclonal gammopathies have recently brought new and unexpected challenges for clinical diagnostics laboratories. In particular, the utilization of therapeutic recombinant monoclonal antibodies has triggered concerns of misdiagnosis of a plasma cell dyscrasia in treated patients. In spite of the fact that monoclonal antibody-based therapies have been in use for several decades, it wasn't until clinical trials with Siltuximab (Centocor), a high-affinity antibody to human IL-6, for treatment of multiple myeloma that a problem was noted. During routine serum protein electrophoresis, the authors detected an apparent IgG heavy-chain immunoglobulin in a patient with a 5-year history of IgDkappa multiple myeloma [10]. They concluded that the interference of monoclonal antibody therapies may be of clinical importance in patients being investigated or monitored for plasma cell dyscrasias. "Individuals receiving monoclonal therapy may be subject to unnecessary follow-up testing, and patients with IgGkappa-producing disease may be misconstrued as experiencing recurrence or resistance to treatment. We suggest that the possibility of false-positive serum protein electrophoresis results needs to be considered, given the increasing use of these agents in clinical practice" [10].

Interestingly, it was demonstrated that although most of the available therapeutic monoclonal antibodies should be seen as monoclonal IgGkappa by immunoelectrophoresis at pharmacological concentrations, only a few antibodies used for non-plasma cell dyscrasia-based diseases formed a visible M-protein on serum protein electrophoresis [11]. Furthermore, addition of infliximab, adalimumab, eculizumab, vedolizumab and rituximab to normal pooled sera revealed no quantifiable M-spikes observed by serum protein electrophoresis at any concentration analyzed in other studies [12]. However, another study found that rituximab, a chimeric anti-CD20 (IgGkappa) monoclonal antibody, is visible on serum protein electrophoresis as a pseudo-paraprotein in patients receiving the therapy [13]. Similarly, therapeutic antibodies like siltuximab, daratumumab and elotuzumab can appear as a small M-protein when used in therapeutic doses [14,15]. In 2015, new monoclonal antibodies were approved for the treatment of relapsed or refractory multiple myeloma - elotuzumab and daratumumab. Elotuzumab is a monoclonal antibody recognizing SLAMF7 (signaling lymphocytic activation molecule F7), a cell surface receptor involved in natural killer cell activation. Daratumumab is a monoclonal antibody that binds to CD38, a transmembrane protein on the surface of myeloma cells responsible for cellular adhesion [16]. Reported interference from these antibodies with M-spike analysis is of special relevance for multiple myeloma patients with a monoclonal protein of the IgGkappa or free kappa light chain subtype because of a co-migration of the therapeutic antibody and the malignant monoclonal antibody may hamper the accurate valuation of the malignant M-protein by serum protein electrophoresis [17]. Appearance of a quantifiable M-protein that has the ability to falsely indicate poor response to therapy in patients with multiple myeloma treated with monoclonal antibodies has been repeatedly confirmed [18,19]. Our own clinical data demonstrate that the treatment of patients with multiple myeloma with elotuzumab or daratumumab often results in the appearance of an alternate IgGkappa monoclonal band in the gamma region on serum protein electrophoresis and immunoelectrophoresis. This suggests that additional approaches to serum electrophoresis are urgently needed to minimize misdiagnosis and overtreatment problems in this patient population.

One approach to overcome the problem of daratumumab co-migration with the malignant M-protein of IgGkappa subtype has been offered by Sebia advertising the Hydrashift 2/4 assay. The assay is based on the results of using the "daratumumab-specific immunofixation electrophoresis reflex assay" (DIRA) which utilizes an *anti-idiotypic* monoclonal antibody targeted to the variable region of daratumumab [17,20]. Binding of this antibody to daratumumab changes the electrophoretic mobility of the therapeutic antibody. Therefore,

pretreatment of a patient serum specimen with this anti-daratumumab antibody shifts the visible bands caused by daratumumab compared to an untreated specimen [18]. Similar assays are currently under development for elotuzumab and isatuximab. These new procedures are anticipated to become commercially available after FDA approval. However, about the available information on the DIRA assay indicates that such additional tests are labor-intensive and require additional lanes of immunofixation, significantly increasing costs for the laboratory, as well as turnaround time.

It is important to note that the free light chain assay is also useful in the diagnosis and therapeutic monitoring of multiple myeloma patients where therapeutic monoclonal antibodies interfere with the immunofixation results. The free light chain assay can be useful in patients who have abnormal free kappa/lambda ratios prior to antibody-based therapy to determine responses particularly in IgG-kappa multiple myeloma [21].

In conclusion, the techniques and approaches used for serum protein electrophoresis have improved significantly in recognition, specificity, and resolution during the past several decades. The more sophisticated techniques of immunofixation, isoelectric focusing, improved gel resolution, serum free light chains, heavy/light analysis and quantification of immunoglobulins are important in the diagnostic work-up of a patient with suspected plasma cell dyscrasias. However, new challenges constantly appear from environmental factors, genetic factors, or novel therapeutic modalities requiring continued evolution and invention of improved diagnostic tools.

## REFERENCES

1. Tiselius A. Electrophoresis of serum globulin. I. *Biochem J* 1937;31:313-17.
2. Tiselius A. Electrophoresis of serum globulin: Electrophoretic analysis of normal and immune sera. *Biochem J* 1937;31:1464-77.
3. Tiselius A. New apparatus for electrophoretic analysis of colloidal mixtures. *Trans Faraday Soc* 1937;33:524-31.
4. Righetti PG. Electrophoresis: the march of pennies, the march of dimes. *J Chromatogr A* 2005;1079:24-40.
5. Willrich MAV, Murray DL, Kyle RA. Laboratory testing for monoclonal gammopathies: Focus on monoclonal gammopathy of undetermined significance and smoldering multiple myeloma. *Clin Biochem* 2017.
6. Tosi P, Tomassetti S, Merli A, et al. Serum free light-chain assay for the detection and monitoring of multiple myeloma and related conditions. *Ther Adv Hematol* 2013;4:37-41.
7. Jenner E. Serum free light chains in clinical laboratory diagnostics. *Clin Chim Acta* 2014;427:15-20.
8. Paolini L. Quantification of beta region IgA paraproteins - should we include immunochemical "heavy/light chain" measurements? Counterpoint. *Clin Chem Lab Med* 2016;54:1059-64.
9. Ermak N, Nguyen-Khoa T, Alyanakian MA. Benefits of new immunoglobulin-derived biomarkers for the diagnosis and follow-up of patients with dysglobulinemia. *Ann Biol Clin (Paris)* 2016;74:597-605.
10. McCudden CR, Voorhees PM, Hainsworth SA, et al. Interference of monoclonal antibody therapies with serum protein electrophoresis tests. *Clin Chem* 2010;56:1897-99.
11. Ruinmans-Koerts J, Verkroost C, Schmidt-Hieltjes Y, et al. Interference of therapeutic monoclonal immunoglobulins in the investigation of M-proteins. *Clin Chem Lab Med* 2014;52:e235-e237.
12. Willrich MA, Ladwig PM, Andreghetto BD, et al. Monoclonal antibody therapeutics as potential interferences on protein electrophoresis and immunofixation. *Clin Chem Lab Med* 2016;54:1085-93.
13. Chakravarthy SN, Ramanathan S, Menon S, et al. Cinderella in Serum Protein Electrophoresis. *Ind J Clin Biochem* 2017.
14. Lonial S, Dimopoulos M, Palumbo A, et al. Elotuzumab Therapy for Relapsed or Refractory Multiple Myeloma. *N Engl J Med* 2015;373:621-31.
15. van de D, Haddad OEl, Axel A, et al. Interference of daratumumab in monitoring multiple myeloma patients using serum immunofixation electrophoresis can be abrogated using the daratumumab IFE reflex assay (DIRA). *Clin Chem Lab Med* 2016;54:1105-09.
16. Sherbenou DW, Mark TM, Forsberg P. Monoclonal Antibodies in Multiple Myeloma: A New Wave of the Future. *Clin Lymphoma Myeloma Leuk* 2017;17:545-54.
17. McCudden CR, Jacobs JFM, Keren D, et al. Recognition and management of common, rare, and novel serum protein electrophoresis and immunofixation interferences. *Clin Biochem* 2017.
18. Murata K, McCash SI, Carroll B, et al. Treatment of multiple myeloma with monoclonal antibodies and the dilemma of false positive M-spikes in peripheral blood. *Clin Biochem* 2016.
19. Rabut E, Castro-Fernandez A, Le Gall V, et al. Case report: serological testing interference of daratumumab (anti-CD38) therapy in multiple myeloma. *Ann Biol Clin (Paris)* 2017;75:351-55.
20. McCudden C, Axel AE, Slaets D, et al. Monitoring multiple myeloma patients treated with daratumumab: teasing out monoclonal antibody interference. *Clin Chem Lab Med* 2016;54:1095-1104.
21. Rosenberg AS, Bainbridge S, Pahwa R, et al. Investigation into the interference of the monoclonal antibody daratumumab on the free light chain assay. *Clin Biochem* 2016;49:1202-04.