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Comparison of the Performance of V8 UltraCE and V8 Nexus Immunodisplacement Assays Using Native Patient Serum Samples

Roni J. Bollag[1], Rachel Siddall[1], Adriana Langer Gramer[2], Shana Marie Williamson Loya[3], Jeff Spencer[3], Gurmukh Singh[1*]

[1]Department of Pathology, Medical College of Georgia, Augusta University, Augusta, GA, USA
[2]Georgia Cancer Center Biorepository, Medical College of Georgia, Augusta University, Augusta, GA, USA
[3]Helena Laboratories, Beaumont, TX, USA
Email: *gusingh@augusta.edu

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Abstract

Background: Capillary electrophoresis and Immunodisplacement assays are used clinically in the evaluation of monoclonal immunoglobulins, similar to assays using gel electrophoresis. In a side-by-side comparison, the performance of a predicate technology and a novel instrument was compared to assess the suitability of the new equipment for clinical use. **Methods:** 340 venous blood serum samples were analyzed by the two methods at two sites in two different countries. The same protocol and reagents were employed at each site. **Results:** The new instrument, V8 UltraCE ID, identified seven additional specimens with monoclonal immunoglobulins, yielding 157 positive results by V8 UltraCE ID and 150 by V8 Nexus ID. The new equipment had the added advantage of a shorter run time. **Conclusions:** The V8 UltraCE ID has superior analytical performance over the existing method and provides greater operational efficiency in analyzing serum samples for the identification of monoclonal gammopathic lesions.

Keywords

Capillary Electrophoresis, Immunodisplacement Assays, Monoclonal Gammopathic Disorders, Oligoclonal Pattern, Multiple Myeloma

1. Introduction

Immunoglobulins are the dominant component of the humoral immune system. Terminally differentiated B lymphocytes, known as plasma cells, produce ten isotypes of immunoglobulins, namely, IgG, IgA, IgM, IgE, and IgD, each with either

kappa or lambda light chains. However, through DNA rearrangement and somatic hypermutation, humans can generate more than 10^{16} different types of immunoglobulin molecules to provide diversity of response against the multiple environmental antigens/challenges, infectious agents, and tumors. This diversity is readily apparent in serum protein gel electrophoresis, where charge differences among immunoglobulin molecules result in a broad area of staining for immunoglobulins [1].

Neoplastic lesions of plasma cells produce a clonal proliferation of one type of cell and secretion of one type of immunoglobulin, termed a monoclonal immunoglobulin. In addition to the classical neoplastic lesions of plasma cells resulting in monoclonal gammopathy of undetermined significance (MGUS), asymptomatic/smoldering multiple myeloma (SMM), and the malignant lesion of multiple myeloma (MM), multiple other pathologies are associated with monoclonal immunoglobulins. The latter include AL amyloidosis, lymphomas, leukemias, and autoimmune disorders [2] [3].

A key component in the diagnosis of MGUS, SMM, and MM is the demonstration of monoclonal immunoglobulins in body fluids, usually serum and urine. In 1% - 3% of the non-secretory MM lesions, no monoclonal immunoglobulins are detectable, and diagnosis is dependent primarily on bone marrow examination [4]. The monoclonal immunoglobulins are usually intact immunoglobulins with variable amounts of free monoclonal light chains. About 15% of the neoplastic plasma cell lesions produce light chains only [3]. Free light chains can be measured by serum free light chain assays (SFLCA), e.g., The Binding Site Freelite® assay. Sebia, Diazyme, Siemens, and other companies offer similar assays [5]. However, none of the available SFLCAs are capable of distinguishing between polyclonal and monoclonal free light chains [6].

As stated above, demonstration of monoclonal immunoglobulins, intact immunoglobulin or monoclonal light chains, or both is critical for the diagnosis of monoclonal gammopathic lesions [2] [3]. Clinical laboratories typically accomplish this through serum protein gel electrophoresis (SPEP) and serum immunofixation electrophoresis (SIFE). An alternative method to gel electrophoresis is Capillary Electrophoresis (CE) and Immunosubtraction or Immunodisplacement (ID). Though gel and capillary electrophoresis employ similar methodologies, capillary electrophoresis allows for a more automated process [2] [7].

While serum protein electrophoresis by capillary electrophoresis is more or less equivalent to gel electrophoresis, immunofixation gel electrophoresis generally has better sensitivity for detecting monoclonal immunoglobulins at lower concentrations. The laboratories employing capillary protein electrophoresis and Immunosubtraction or Immunodisplacement for the detection of monoclonal immunoglobulin also host gel-based immunofixation electrophoresis for selective instances of low concentration monoclonal immunoglobulins [2]. While capillary electrophoresis and Immunosubtraction or Immunodisplacement are more suitable for automation, gel-based immunofixation electrophoresis has also been au-

tomated [8].

The more open architecture of gel electrophoresis allows easier modification of the process for enhancing the detection of unusual patterns and for improving sensitivity [9]-[12]. A notable example of the latter is the use of undiluted serum in immunofixation for enhanced sensitivity detection of free monoclonal light chains, namely, FLC Modified SIFE. This method has the sensitivity of mass spectrometry without the need for special equipment or technical expertise. QUIET and FLC Modified SIFE not only allow detection but quantification of monoclonal free light chains, for which there is no other practical method that is easier than mass spectrometry [13]-[16].

In this study, we evaluated Immunodisplacement on a new CE instrument, the V8 UltraCE, against the existing FDA-cleared V8 Nexus Immunodisplacement assay for identification of monoclonal immunoglobulins.

2. Methods

Residual serum samples from venous blood collected for clinical testing were employed at two sites for comparison testing of the two instruments. The samples were convenience-based. Most of the specimens were post-treatment; however, the deidentification of the specimens precluded exact numbers and the status of the specimens. The manufacturer did not have any role in the selection of specimens; however, two of the authors are employees of the sponsor and were involved in testing, data analysis, and reviewing the manuscript. The clinical status of the samples was known, and most samples contained monoclonal immunoglobulins. Each of the testing sites followed its institutional ethical protocols in compliance with the Helsinki principles; this included deidentification of samples for patient security. The equipment and test kits were provided by Helena Laboratories (Beaumont, TX, USA) and Helena Biosciences Europe (Gateshead, UK) [7].

The two sites used identical operating procedures and the same lot of test kits for analyzing the specimens. Each site employed its own personnel and operated within its routine work environment. The two instruments were sited near each other to ensure uniformity of ambient temperature and other operating conditions. The specimens were run in the V8 Nexus instrument, and at the completion of the run, the same tubes were loaded onto the V8 UltraCE. The specimens were run on multiple days; however, each specimen tube was processed on the same day on the two instruments. Results from the two instruments were interpreted by the same person. The reading could not be blinded to the type of instrument due to differences in the output formats. Reading of the results was geared towards the identification of monoclonal immunoglobulins and their isotypes. More than one immunoglobulin of restricted mobility was noted in some patients. This could have been from the natural occurrence of a biclonal pattern in about 1% - 3% of monoclonal gammopathic disorders. More commonly, multiple bands were noted post-treatment, especially treatment with autologous stem cell transplantation. Immunoglobulin bands of restricted mobility resulting from the treatment of patients with therapeutic monoclonal immunoglobulins were not segregated. No attempt

was made to identify the therapeutic immunoglobulin preparation; only the presence of restricted heterogeneity immunoglobulin was noted. Test results from each site were submitted to a central location for analysis.

Samples were excluded from analysis based on the following exclusion criteria: insufficient sample volume, capillary migration failures, nonspecific binding or interference, high background signal, weak trace signal, instrument malfunction, ID trace quality, equivocal or uninterpretable ID traces, and insufficient time/specimen for repeat testing.

Results from the two instruments at each site were tabulated and compared. The same was done for the pooled results from two sites. The comparison of the recorded results was geared towards noting concordance, or lack thereof, of results. A 2 × 2 chi-square test did not reveal a statistically significant difference in the performance of two instruments.

3. Results

The demographic data and the number of specimens excluded at each of the sites are given in Table 1. After exclusions, 340 specimens were analyzed between the two sites. Due to constraints in the logistics of this non-inferiority validation, some items were not available for reporting in this section. As noted earlier, deidentification of specimens precluded comparison with results of a gel electrophoresis testing method.

Site 2 had 13 samples with no demographics. For Site 2, statistics for sex and age were calculated for 197 of the 210 specimens.

Table 1. Demographic data and the number of specimens excluded at each of the sites.

	Site 1	Site 2
Number	128	212
Male	67	77
Female	61	122
No Demographics	0	13
Age Range-Years (Mean)	34 - 90 (69.3)	5 - 99 (66.0)
Min Age-Years	34	5
Max Age-Years	90	99
Mean Age-Years	69.3	66
Exclusions	17	1

Table 2 shows the results of the pooled data from two sites. Three hundred forty samples were analyzed in total. The V8 Nexus identified 150 samples containing at least one monoclonal band versus 157 identified by the V8 UltraCE Immunodisplacement assay.

Table 2. Results of the pooled data from two sites.

Sites 1 and 2, Pooled Data		
Immunotypes	V8 Nexus ID	V8 UltraCE ID
No Monoclonal	190	183
IgG Kappa	67	68
IgG Lambda	33	35
IgA Kappa	14	14
IgA Lambda	2	3
IgM Kappa	5	7
IgM Lambda	1	1
Free Kappa	1	1
Free Lambda	3	3
2X IgG Kappa	6	6
2X IgA Kappa	2	2
2X IgA Lambda	1	1
2X IgM Kappa	1	1
2X Free Kappa	0	1
2X Free Lambda	1	1
IgG Kappa/IgG Lambda	3	3
IgG Kappa/IgA Kappa	1	1
IgG Kappa/IgM Lambda	1	0
IgG Lambda/Free Lambda	1	1
IgA Lambda/Free Lambda	1	1
IgM Kappa/IgM Lambda	1	1
IgM Kappa/Free Kappa	1	1
3X IgG Kappa	1	1
3X IgA Lambda	1	1
IgG Kappa/2X IgG Lambda	1	1
IgG Kappa/IgM Lambda/IgM Kappa	0	1
3X IgG Lambda/Free Lambda	1	1
Total Number of Specimens	340	340
Monoclonal Positive Samples	150	157

Explanatory Notes: 2X or 3X represents multiple bands of a given type. One IgM clone was missed by V8 UltraCE, which was detected by the older method of V8 Nexus, indicated by orange shading in Table 2. However, overall, more clones of IgM were detected by V8 UltraCE than by V8 Nexus. Ten clones of different types were missed by V8 Nexus but were detected by V8 UltraCE, shown by yellow shading in Table 2.

4. Discussion

An earlier study compared the performance of Helena V8 UltraCE and Sebia Capillarys and demonstrated higher resolution with the Helena instrument [7]. The higher resolution was likely the result of a shorter, narrower capillary, allowing higher voltage and active cooling of the capillary during the separation run. The shorter capillary length also allowed for a shorter run time. These characteristics also apply to the comparison of Helena V8 UltraCE and V8 Nexus, in general, and a shorter run time in particular. Active cooling of the capillary during high voltage run likely contributed to better performance of Helena V8 UltraCE, especially detection of monoclonal bands at a lower concentration. Protein monitoring at multiple wavelengths in Helena V8 UltraCE also allows distinction of proteins from other UV-absorbing materials like radiocontrast media and antibiotics.

The V8 UltraCE Immunodisplacement assay positively identified restricted heterogeneity bands in 157 specimens versus 150 specimens with restricted heterogeneity bands identified by the V8 Nexus Immunodisplacement assay, demonstrating the increased detection capabilities of the V8 UltraCE Immunodisplacement assay. Two possible reasons for the discordance in the samples above are comigration of monoclonals interfering with immunotyping and the enhanced resolving capabilities of the V8 UltraCE Immunodisplacement assay compared to the V8 Nexus Immunodisplacement assay.

It warrants emphasis that serum free light chain assay is not a replacement for serum immunofixation electrophoresis/Immunodisplacement/Immunosubtraction assays, nor for urine immunofixation electrophoresis. None of the tests for serum free light chains can distinguish between polyclonal and monoclonal free light chains [17]. It warrants mention that while it could be done, capillary electrophoresis and Immunosubtraction or Immunodisplacement are not commonly used for examination of urine. As discussed earlier, gel electrophoresis allows easier modification of standard procedures, and such a modification of the urine immunofixation electrophoresis method improved the detection rate of monoclonal free light chain in urine by 18.2% [18].

Multiple bands observed in some of the specimens are likely attributable to the oligoclonal pattern seen in patients following autologous stem cell transplantation. A similar oligoclonal pattern may also arise following chemotherapy [19].

The V8 UltraCE Immunodisplacement assay not only demonstrates substantial equivalence to the V8 Nexus Immunodisplacement assay in the detection of monoclonal proteins in serum but also provides a more powerful detection method than the older instrument. Higher sensitivity assays for the detection of monoclonal immunoglobulins in serum and urine are important in avoiding bone marrow examination while trying to detect minimal residual disease in multiple myeloma patients [20]-[24].

Limitations of this study include a lack of clinical details, e.g., whether the specimens were pretreatment or post-treatment, and what treatment was applied. The latter has implications for reading the results if therapeutic monoclonal antibodies

were used in treatment. The statistical analysis was limited to the chi-square test, which did not reveal a significant difference in the performance of the two methods. The intent of the study was a comparative non-inferiority analysis to validate the performance of the V8 Ultra CE versus the predicate FDA 510(K)-cleared V8 Nexus platform. An additional weakness of the study is the lack of orthogonal analysis for specimens in which the results of the two methods were at variance.

5. Conclusion

The V8 UltraCE method has better sensitivity, though the difference from the older method using V8 Nexus was not statistically significant. The shorter run time with V8 UltraCE methods provides a logistical advantage.

Statement

GS serves as a consultant to Helena Laboratories, Sebia Inc., Diazyme Laboratories, Nexicart-2 IRC, and the Warm Springs, GA, medical center. GS and RB have applied for a patent for the FLC-Modified SIFE method.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Schroeder, H.W. and Cavacini, L. (2010) Structure and Function of Immunoglobulins. *Journal of Allergy and Clinical Immunology*, **125**, S41-S52. https://doi.org/10.1016/j.jaci.2009.09.046

[2] Keren, D.F. (2024) Electrophoresis and Mass Spectrometry. Monoclonal Gammopathies and Protein Disorders. ASCP Press.

[3] Singh, G. (2020) Serum and Urine Protein Electrophoresis and Serum-Free Light Chain Assays in the Diagnosis and Monitoring of Monoclonal Gammopathies. *The Journal of Applied Laboratory Medicine*, **5**, 1358-1371. https://doi.org/10.1093/jalm/jfaa153

[4] Migkou, M., Avivi, I., Gavriatopoulou, M., Cohen, Y.C., Fotiou, D., Kanellias, N., *et al*. (2020) Clinical Characteristics and Outcomes of Oligosecretory and Non-Secretory Multiple Myeloma. *Annals of Hematology*, **99**, 1251-1255. https://doi.org/10.1007/s00277-020-03984-w

[5] Bradwell, A.R. (2015) Serum Free Light Chain Analysis plus Hevylite. 7th Edition, The Binding Site Group Ltd.

[6] Singh, G. (2025) Screening for Monoclonal Gammopathy of Undetermined Significance Is Contraindicated. *British Journal of Haematology*, **207**, 43-45. https://doi.org/10.1111/bjh.20175

[7] Delanghe, J.R., Godefroid, M. and Maenhout, T. (2026) Validation Study of the Helena V8 UltraCE Capillary Elecrophoresis Analyser. *Clinical Chemistry and Laboratory Medicine*, **64**, 1399-1403.

[8] Singh, G., Saldaña, E.J., Spencer, J. and Bollag, R.J. (2025) Automated Detection of Free Monoclonal Light Chains by Enhanced-Sensitivity Modified Immunofixation Electrophoresis with Antisera against Free Light Chains. *Laboratory Medicine*, **56**, 536-540. https://doi.org/10.1093/labmed/lmaf014

[9] Larsen, R., Allen, S., Thompson, T.Z., Bollag, R. and Singh, G. (2019) Challenges in Interpreting Multiple Monoclonal Bands on Serum Protein Electrophoresis and Serum Immunofixation Electrophoresis: An Illustrative Case Report. *The Journal of Applied Laboratory Medicine*, **4**, 455-459. https://doi.org/10.1373/jalm.2018.027938

[10] Nwogbo, O.V., Jin, Y., Sliker, T., Wilhite, D. and Singh, G. (2021) Analysis of Multiple Bands on Serum Protein Immunofixation Electrophoresis: Challenge in Interpretation of Clonality in a Patient with Light Chain-Predominant Multiple Myeloma. *Laboratory Medicine*, **52**, 503-508. https://doi.org/10.1093/labmed/lmab001

[11] Ullah, A., Omar, N., Savage, N.M., Bollag, R.J. and Singh, G. (2021) Immune Subtraction for Improved Resolution in Serum Protein Immunofixation Electrophoresis and Antibody Isotype Determination in a Patient with Autoantibody. *Practical Laboratory Medicine*, **26**, e00240. https://doi.org/10.1016/j.plabm.2021.e00240

[12] Omar, N., Madwani, K., Moideen, P., Manthei, D.M., Keren, D.F. and Singh, G. (2021) Accurate Quantification of Monoclonal Immunoglobulins Migrating in the Beta Region on Protein Electrophoresis. *Laboratory Medicine*, **53**, 138-144. https://doi.org/10.1093/labmed/lmab055

[13] Singh, G. and Bollag, R. (2020) Quantification by Ultrafiltration and Immunofixation Electrophoresis Testing for Monoclonal Serum Free Light Chains. *Laboratory Medicine*, **51**, 592-600. https://doi.org/10.1093/labmed/lmaa012

[14] Wilhite, D., Arfa, A., Cotter, T., Savage, N.M., Bollag, R.J. and Singh, G. (2021) Multiple Myeloma: Detection of Free Monoclonal Light Chains by Modified Immunofixation Electrophoresis with Antisera against Free Light Chains. *Practical Laboratory Medicine*, **27**, e00256. https://doi.org/10.1016/j.plabm.2021.e00256

[15] Giles, H.V., Wechalekar, A. and Pratt, G. (2022) The Potential Role of Mass Spectrometry for the Identification and Monitoring of Patients with Plasma Cell Disorders: Where Are We Now and Which Questions Remain Unanswered? *British Journal of Haematology*, **198**, 641-653. https://doi.org/10.1111/bjh.18226

[16] Giles, H.V., Cook, M.A., Drayson, M.T., Cook, G., Wright, N.J., North, S.J., *et al.* (2022) Redefining Nonmeasurable Multiple Myeloma Using Mass Spectrometry. *Blood*, **139**, 946-950. https://doi.org/10.1182/blood.2021013794

[17] Singh, G. (2025) Use and Misuse of Serum Free Light Chain Assay: Challenging the Paradigms. *Archives of Clinical and Biomedical Research*, **9**, 471-482. https://doi.org/10.26502/acbr.50170490

[18] Singh, G., Arinze, N., Manthei, D.M., Plapp, F.V. and Bollag, R.J. (2023) Urine Protein Immunofixation Electrophoresis: Free Light Chain Urine Immunofixation Electrophoresis Is More Sensitive than Conventional Assays for Detecting Monoclonal Light Chains and Could Serve as a Marker of Minimal Residual Disease. *Laboratory Medicine*, **54**, 527-533. https://doi.org/10.1093/labmed/lmac155

[19] Singh, G. (2017) Oligoclonal Pattern/Abnormal Protein Bands in Post-Treatment Plasma Cell Myeloma Patients: Implications for Protein Electrophoresis and Serum Free Light Chain Assay Results. *Journal of Clinical Medicine Research*, **9**, 671-679. https://doi.org/10.14740/jocmr3049w

[20] Singh, G. (2025) Monoclonal Gammopathy. *British Journal of Haematology*, **206**, 1857-1858. https://doi.org/10.1111/bjh.20059

[21] Szalat, R., Anderson, K. and Munshi, N. (2024) Role of Minimal Residual Disease Assessment in Multiple Myeloma. *Haematologica*, **109**, 2049-2059. https://doi.org/10.3324/haematol.2023.284662

[22] Foureau, D.M., Paul, B.A., Guo, F., Lipford, E.H., Fesenkova, K., Tjaden, E., *et al.* (2023) Standardizing Clinical Workflow for Assessing Minimal Residual Disease by Flow

Cytometry in Multiple Myeloma. *Clinical Lymphoma Myeloma and Leukemia*, **23**, e41-e50. https://doi.org/10.1016/j.clml.2022.10.008

[23] Bai, Y., Orfao, A. and Chim, C.S. (2018) Molecular Detection of Minimal Residual Disease in Multiple Myeloma. *British Journal of Haematology*, **181**, 11-26. https://doi.org/10.1111/bjh.15075

[24] Singh, G. (2026) Serum Free Light Chain Concentration and Ratio-Based Criterion for Myeloma-Defining Event, in Its Current Version, Is Untenable. *British Journal of Haematology*, **208**, 1555-1563. https://doi.org/10.1111/bjh.70427