V8 Immunodisplacement Procedure

Cat. No. 1803C

INTENDED USE

The V8 Immunodisplacement method is designed for the detection and the characterization of monoclonal proteins, including immunoglobulins IgG, IgA, IgM, kappa (bound) and lambda (bound) light chains, in human serum with the Helena V8 Capillary Electrophoresis System.

SUMMARY

The demand for Immunodisplacement in the clinical laboratory is primarily for the detection of monoclonal gammopathies. A monoclonal gammopathy is a primary disease state in which a single clone of plasma cells produces elevated levels of an immunoglobulin of a single class and type. Such immunoglobulins are referred to as monoclonal proteins, M-proteins or paraproteins. Their presence may be of a benign nature or of uncertain significance. In some cases, they are indicative of a malignancy, such as multiple myeloma or Waldenström's macroglobulinemia. Differentiation must be made between polyclonal and monoclonal gammopathies, as polyclonal gammopathies are a secondary disease state due to clinical disorders such as chronic liver disease, collagen disorders, rheumatoid arthritis and chronic infection. The method used in conjunction with the V8 Serum Protein SPE Kit designed for serum protein separation into six major fractions in alkaline buffer. The electrophoretograms of separated proteins mixed with individual specific antisera are evaluated visually to detect the presence of specific reactions with the suspect monoclonal proteins. The test results are to be used in conjunction with clinical findings and other laboratory tests.

PRINCIPLE

The V8 Immunodisplacement Kit is intended for the separation and identification of monoclonal gammopathies by capillary zone electrophoresis. This technique separates proteins on the basis of their net charge in an alkaline buffer solution in combination with their differing interaction with the wall of the silica capillary. Immunotyping of gammaglobulins is achieved by testing aliquots of sample with a panel of monospecific antisera. The complex formed by the test antisera and its target protein has a modified migration profile and is therefore displaced from the standard serum protein electropherogram. By comparing the results from the test panel with a reference analysis, the immunoglobulin type present can be determined by the specific removal or reduction of the abnormal spike from the CE electropherogram.

REAGENTS

1. **Antisera to Human IgG, IgA, IgM, Kappa Light Chain and Lambda Light Chain**

   Ingredients: Antisera vials in the kit contain monospecific antisera to human immunogobulin heavy, chains, IgG, IgM, IgA and to human light chains, Kappa and Lambda. The antisera have been prepared in goat. Each vial of antiserum contains a stabilizer and ProClin as a preservative.

   WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. Incidental contact with or ingestion of buffers or reagents containing ProClin can cause irritation to the skin, eyes or mouth. Use good laboratory practices to reduce exposure. Seek medical attention if symptoms are experienced.

   Preparation of Use: The antisera are ready for use as packaged.

   Storage and Stability: The antisera should be stored at 2 to 8°C and are stable until the expiration date indicated on the vial. Opened bottles placed in the reagent bottle area of the V8 instrument are stable for 4 weeks. Ensure antisera vials are re-capped when not in use to minimize evaporative losses. **DO NOT FREEZE.**

   Signs of Deterioration: Coloration or cloudiness of the antisera may be indicative of bacterial contamination.

INSTRUMENT

The Helena V8 Capillary Electrophoresis System must be used to analyze the sample. Refer to the Operator Manual for detailed instructions.

SPECIMEN COLLECTION AND HANDLING

Specimen: For serum protein analysis, freshly collected serum is the specimen of choice as plasma samples will contain a large fibrinogen peak between the beta and gamma fractions.

Storage and Stability: Samples can be stored at 2 to 8°C for up to 7 days and up to 6 months at -20°C. If samples are to be stored frozen, they should be refrigerated immediately and frozen within 8 hours of collection. Storing samples at 2 to 8°C can result in protein degradation, particularly, but not exclusively of complement fractions. Consequently, after 7 days storage at 2 to 8°C, detection of a distinct $beta_2$ region may no longer be possible. DO NOT store samples at room temperature - the sample will degrade rapidly. Samples which contain cryoglobulins may become viscous or turbid after refrigeration or freezing. It is advisable to warm these samples to room temperature before analysis.

PROCEDURE

Materials provided: The following materials are provided:

Sample Test Size	Cat. No.
50	1803C
Anti-human IgG antisera	1 vial
Anti-human IgA antisera	1 vial
Anti-human IgM antisera	1 vial
Anti-human kappa antisera	1 vial
Anti-human lambda antisera	1 vial

Materials provided but not contained in the above kit:

Item	Cat. No.
V8 Velocity Analyzer	1800
V8 Nexus CE Analyzer	1825
V8 Storage Buffer	1831
V8 Maintenance Buffer	1832
V8 Serum Protein SPE Kit	1805
V8 Clinical Waste Drawer Inserts	1820

Materials required but not provided:

Item

Uncapped primary tubes

STEP BY STEP PROCEDURE

These instructions are for standard mode versus touch screen.

(For correct installation of all consumables, please refer to the Operator Manual)

1. Ensure that the waste container drawer is on-board.
2. Before switching on the V8, ensure that the Storage Buffer, Maintenance Buffer and disposable cups are on-board and in their correct positions.
3. Launch Platinum and begin a new V8 session. In Platinum, select "V8 SYSTEM" from the drop down menu, click "SELECT DEFAULT METHOD" and ensure the relevant Immunodisplacement assay is selected. For reflex testing or individual test ordering, please refer to the Operator Manual.
4. Switch on the V8 as instructed in the Operator Manual.
5. To conduct Immunodisplacement testing, install the relevant Serum Protein Buffer and Diluent if required.
6. Ensure that the Immunodisplacement reagent barcode information is loaded into Platinum by selecting "V8 SYSTEM" from the drop down menu. Click "DEFINE REAGENTS" and ensure the barcode information for each antisera type has been entered and that the location of the reagent information corresponds with the intended vial location on-board the V8.
7. Uncap the antisera vials and place them in the reagent bay of the V8.
8. When the V8 is ready to accept samples for operation, the instrument will pulse red.
9. For samples that have been ordered for Reflex testing and are NOT already on-board the V8, place each primary sample tube into the sample rack, ensuring that the barcode is clearly visible through the rack window.
10. Load sample racks onto the left-hand side of the V8 sample transport area and close the lid as instructed in the Operator Manual.
11. The V8 will automatically commence analysis of all loaded samples, and the results will be transferred to Platinum.
12. After analysis, and if required, initiate V8 shut-down mode by switching off the instrument as instructed in the Operator Manual.

    NOTE: It is important that the V8 is post-condi tioned correctly at the end of the day.
13. Refer to INTERPRETATION OF RESULTS once data is generated.

INTERPRETATION OF RESULTS

Abnormal Sample Results

The majority of monoclonal proteins migrate in the cathodic, gamma region of the protein pattern, but due to their abnormal nature, they may migrate anywhere within the globulin region on capillary electrophoresis.

Immunodisplacement is a subtractive analytical technique and the abnormal protein is identified by noting which antisera types react with it, resulting in the originally identified peak being reduced or removed in the test CE electropherograms. Care must be taken when analyzing results as normal polyclonal immunoglobulins will be removed from the trace as well as abnormal paraproteins. Attention must therefore be paid to the shape of the test electropherograms as well as their relative peak areas.

Normal Sample Results

A normal sample will present a gamma region with no visible peaks or asymmetries. Subsequent Immunotyping of a normal sample is dealt with in point (6) in the LIMITATIONS section.

Detection of Low Level Monoclonal and Biclonal Peaks

Immunodisplacement identifies target immunoglobulins by removing them from an abnormal serum protein trace. Low concentration monoclonal/biclonal peaks may require a closer examination in order to assess whether they have been removed from any given trace. This can be achieved by reducing the scale of the axes or zooming in using the tools available in the trace window. The trace image produced can be reported using the “copy trace image” tool if required. These functions are dealt with in full in the V8 Capillary Electrophoresis System Operator Manual.

It is recommended that any difficult to classify samples or samples with monoclonal components below 25 mg/dL be further analyzed by gel IFE.

Low concentration samples may be reanalyzed using an increased dilution (please refer to override dilutions in the Operator Manual).

LIMITATIONS

1. **Reaction with Kappa or Lambda light chain antisera but no reaction with IgG, IgA or IgM heavy chain antisera.** Samples showing this pattern may either have a free light chain monoclonal gammopathy or they may have an IgD or IgE monoclonal protein. In this situation, the sample should be further analyzed by gel immunofixation with IgD and IgE IFE antisera. Failure to obtain a reaction with IgD or IgE antisera would be indicative of free light chain disease.
2. **Band in gamma region showing no reactivity with ID antisera.** C Reactive Protein (CRP) may be detected in patients with acute inflammatory response 2-3. Elevated alpha$_1$ antitrypsin and haptoglobin are supportive evidence for CRP.
3. **Non-reactivity with Kappa and Lambda antisera.** Occasionally a sample will have a reaction with a heavy chain antiserum but no light chain reaction is obvious. In this situation, the following need to be ruled out:
   a. Heavy chain disease,
   b. Very high concentrations of light chains, leading to antigen excess,
   c. Low concentrations of light chains,
   d. Atypical light chain molecule that does not react with the antiserum,
   e. Light chains with ‘hidden’ light chain determinants, as sometimes seen with IgA and IgD immunoglobulins.
4. **Cross Reactivity of Antisera.** Due to the modified nature of Immunodisplacement antibody molecules, antibody-antigen reactions performed on capillary can result in some antisera cross reacting with monoclonal components present in a sample. Cross reactivity, which is a rare occurrence, may lead to the clinical conclusion of a biclonal gammopathy. If cross reactivity of antisera

is suspected further testing using gel immunofixation may be necessary to confirm results. There is no risk of false negative results as a consequence of cross reactivity. The Immunodisplacement antisera are capable of completely removing monoclonal peaks of < 30 g/L from serum samples. Gammaglobulins in large excess of these concentrations may not be entirely removed from the trace, rendering interpretation of the results difficult. In such cases, it is advised that the original patient sample is diluted with isotonic saline to reduce the protein concentration to a level at which complete displacement can occur and the Immunodisplacement assay procedure is repeated.

5. **Detection of Low Level Monoclonal and Biclonal Peaks.** The Immunodisplacement identifies target immunoglobulins by removing them from an abnormal serum protein trace. Low concentration monoclonal/biclonal peaks may require a closer examination in order to assess whether they have been removed from any given trace. This can be achieved by reducing the scale of the axes or zooming in using the tools available in the trace window. The trace image produced can be reported using the "copy trace image" tool if required. These functions are dealt with in full in the V8 Capillary Electrophoresis System Operator Manual. It is recommended that any difficult to classify samples or samples with monoclonal components below 0.25 g/L be further analyzed by gel IFE.
6. **Immunodisplacement in Normal Samples.** The antisera used in Immunodisplacement are polyclonal antisera against specific human immunoglobulin subtypes. As such any antigen/antibody binding will take place against both the targeted immunoglobulins of monoclonal origin and against polyclonal immunoglobulins in the sample being analyzed. The appearance of differences in the traces of normal patient samples is due to the removal of the polyclonal immunoglobulins from the electrophoretogram. This is most evident in those traces for anti-IgG and anti-IgKappa antisera due to the significantly larger concentrations of these immunoglobulins in comparison to the others e.g., IgA, IgM present in the sample. Although there is removal of small amounts of polyclonal IgA, IgM etc. the visual effect they have on the electrophoretogram is minimal.

PERFORMANCE CHARACTERISTICS

1. **Prozone Effects (V8 Immunodisplacement)**
   The effect described as prozoning as seen in gel electrophoresis can be described as a phenomenon in which mixtures of specific antigen and antibody do not agglutinate or precipitate visibly because of an excess of either antibody or antigen. In the case of the V8 Immunodisplacement (ID) assay, an excess of antigen can be visualized by the appearance of residual protein from an apparently reduced monoclonal peak. The target capacity of the ID antisera is around 3 g/dL. Any monoclonal that is at a greater concentration will show residual protein in the resultant trace. Diluting samples with high antigen concentrations can reduce or eliminate this effect.
2. **Method Comparison Study Summary of Results**
   The Helena V8 was compared against the SPIFE IFE System using a total of 131 normal and abnormal samples in three separate facilities. The results can be see in the table below:

Qualitative Results	Total Number	Complete Agreement	Partial Agreement	Incomplete Agreement	Monoclonal Concentration Ranges (g/L)
Normal	22	22	0	0	N/A
IgG κ	36	36	0	0	0.35 - 4.78
IgG λ	23	23	0	0	0.47 - 4.39
IgA κ	9	8	1	0	0.27 - 4.09
IgA λ	11	11	0	0	0.25 - 4.09
IgM κ	18	18	0	0	0.30 - 3.10
IgM λ	5	5	0	0	0.80 - 2.80
Biclonal	2	2	0	0	0.40 - 0.70
Oligoclonal	1	1	1	0	1.50
Other	4	4	0	0	-
Grand Total	131	130	1	0	N/A

3. **Precision/Reproducibility**
   Eight samples were used for reproducibility study, including one normal sample and seven pathological samples: monoclonal subtypes IgG κ (n=1), IgG λ (n=1), IgA κ (n=1), IgA λ (n=1), IgM κ (n=1), IgM λ (n=1). The total Immunoglobulin levels were between 2.0 and 22.5 g/L. Each sample was analyzed in six replicates and the run was repeated with three different lots of antisera. According to the identified monoclonal protein characterization, replicates of each sample showed 100% concordant and reproducible results within each run and between different lots of antisera.

INTERFERENCES

No interference was observed in six pathological samples (IgG κ, IgG λ, IgA κ, IgA λ, IgM κ, IgM λ ranging from 1.3 g/L to 43.0 g/L monoclonal protein concentrations) spiked with hemoglobin up to 0.17 g/dL; 16.05 mg/L indirect bilirubin; 15.9 mg/dL direct bilirubin; triglycerides at 386, 669, 1991 mg/dL; 317 IU/mL RF at 0 mg/dL; up to 16.28 mmol/L cholesterol.

SENSITIVITY

The antisera is able to remove protein up to 3 g/dL and is sensitive down to approximately 0.025 g/dL.

Six pathological samples comprising each monoclonal isotype IgG κ (51 g/L), IgG λ (37 g/L), IgA κ (69 g/L), IgA λ (61 g/L), IgM κ (42 g/L), IgM λ (69 g/L) were diluted with a normal sample until a sample was produced with a monoclonal peak of 0.75 g/L. These initial diluted samples were further serially diluted to produce a range of monoclonal concentrations of 0.45, 0.35, 0.25, 0.20, and 0.15 g/L. These dilutions were analyzed by the V8 System to show the decreasing appearance of the monoclonal peak. The data demonstrated the claimed detection limit of 0.25 g/L.

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