V8 Serum Protein SPE Procedure

Cat. No. 1805

INTENDED USE

The V8 Serum Protein SPE method is intended for the separation and quantitation of serum proteins on the Helena V8 Capillary Electrophoresis System.

SUMMARY

Serum contains over one hundred individual proteins, each with a specific set of functions which are subject to specific variation in concentration under different pathological conditions.[1]

Since the introduction of moving-boundary electrophoresis by Tiselius[2] and the subsequent use of zone electrophoresis, serum proteins have been fractionated on the basis of their charge at a particular pH.

PRINCIPLE

The V8 Serum Protein SPE Kit utilizes an alkaline buffer to separate serum proteins into 6 main classes (albumin, alpha$_1$ globulin, alpha$_2$ globulin, beta$_1$ globulin, beta$_2$ globulin and gamma globulin). Each of the electrophoretic zones, with the exception of albumin, normally contains at least 2 components. The relative proportions of these fractions have proven useful aids in the diagnosis and prognosis of certain disease states.[3-5]

REAGENTS

1. **V8 SPE Buffer**
   Ingredients: The buffer contains an antimicrobial**.**
   WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST
   Preparation for Use: The reagent is ready for use as packaged.
   Storage and Stability: The buffer should be stored at 15 to 30°C and is stable until the expiration date indicated on the package. The buffer is stable for six months when stored at 15 to 30°C. **DO NOT FREEZE.**
   Signs of Deterioration: The buffer should be a clear, colorless solution. Discard if the buffer becomes cloudy.
2. **V8 SPE Diluent**
   Ingredients: The diluent contains buffers and an antimicrobial.
   WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT INGEST
   Preparation for Use: The reagent is ready for use as packaged.
   Storage and Stability: The diluent should be stored at 15 to 30°C and is stable until the expiration date indicated on the package. The diluent, when capped, is stable for six months when stored at 15 to 30°C . **DO NOT FREEZE.**
   Signs of Deterioration: The diluent should be a clear, colorless solution. Discard if the diluent becomes cloudy.

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

Freshly collected serum is the specimen of choice as plasma samples will contain a large fibrinogen band between the beta and gamma fractions. Samples can be stored at 2 to 8°C for up to 7 days and up to 1 month at -20°C. If samples are to be stored frozen, refrigerate immediately and freeze 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 stored 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.

INTERFERING FACTORS

1. Hemolysis may cause false elevation in the alpha$_2$ and beta fractions.
2. Inaccurate results may be obtained on specimens left uncovered, due to evaporation.
3. The presence of radiopaque contrast media can cause the alpha$_2$ and beta regions of the electropherogram to appear elevated.

USAGE MATRIX

The number of tests per bottle of buffer may be less than 500 when system throughput is less than 120 tests per session.

Approximate yield per bottle according to number of tests is given below:

120+ tests per session:	500 tests per bottle
104 tests per session:	480 tests per bottle
64 tests per session:	400 tests per bottle
40 tests per session:	320 tests per bottle

PROCEDURE

Materials provided: The following materials needed for the procedure are contained in the V8 Serum Protein SPE Kit (Cat. No. 1805).

V8 SPE Buffer (1 bottle)
V8 SPE Diluent (3 x 20 mL)
Filter (1)
Sample cups (4 pkg)

Materials needed but not contained in the kit:

Item	Cat. No.
V8 Nexus CE Analyzer	1825
V8 SP Normal Control	1810
V8 SP Abnormal Control	1811
V8 Storage Buffer	1831
V8 Maintenance Buffer	1832
V8 Clinical Waste Drawer Inserts	1820

OPTIONAL ITEMS

Item	Cat. No.
V8 SPE Buffer Kit	1835
V8 Immunodisplacement Kit	1803

STEP-BY-STEP METHOD

For correct installation of all consumables, please refer to the V8 Operator Manual or Platinum Touchscreen Operator Manual.

1. Before switching on the V8, ensure the Storage Buffer, Maintenance Buffer, disposable cups and waste container drawer are onboard and in the correct positions.
2. Switch on the V8 instrument, launch Platinum, and begin a new V8 session. In Platinum, ensure that SPE is selected as the default method or use test ordering to assign the SPE assay to specific samples. **NOTE: To run the instrument equiped with the Fast CE capillaries, select the Fast CE method.**
3. If prompted by the V8 instrument, install the V8 SPE Buffer bottle into the fluid bottle compartment and verify that it is in the correct position. **NOTE: For correct installation, apply a fresh filter unit to the inlet pipe of the buffer bottle connector before installing it on the V8 instrument.**
4. Installation of the V8 SPE Buffer bottle will automatically open a prompt in Platinum. Scan or enter the barcode number into the buffer position field matching the bottle position.
5. Scan or enter the barcode number for the V8 SPE Diluent into Platinum, and verify that the location of the reagent information corresponds with the intended vial location onboard the V8 instrument.
6. Remove the lid from the V8 SPE Diluent, and ensure that the bottle is placed correctly in the reagent bottle area.
7. When the V8 instrument is ready to accept samples for operation, the instrument will pulse red.
8. Load the primary tubes into sample racks, ensuring that the barcodes are visible through the rack window.
9. Load the sample racks onto the left-hand side of the V8 sample transport area, and close the lid.
10. The V8 instrument will automatically commence analysis of all loaded samples, and the results will be transferred to Platinum.
11. After analysis, if required, initiate V8 shut-down mode by switching off the instrument.
    NOTE: The V8 instrument must be post-conditioned correctly at the end of the day.

QUALITY CONTROL

V8 SP Normal and Abnormal Controls (Cat. No. 1810 and 1811) can be used to verify all phases of the Serum Protein procedure. Refer to the package insert for the appropriate assay values.

INTERPRETATION OF RESULTS

It is recommended that any evaluation of the CE traces is performed against normal values produced for this method in each individual laboratory. For a complete review of serum protein evaluation, see Ritzmann, S.E.,1982.[5] Studies show that the values are the same for both males and non-pregnant females. Some differences are seen in pregnant females at term and women on oral contraceptives. Age has some effect on normal levels. Cord blood has a decreased total protein, albumin, $alpha_2$ and beta fractions; slightly increased $alpha_1$ and normal or increased gamma fraction (largely of maternal origin). The gamma globulins drop rapidly until about 3 months of age, while other fractions have reached adult levels by this time. Adult levels of the gamma globulins are not reached until 10-16 years of age. The albumin decreases and beta globulin increases over the age of 40.

LIMITATIONS

Due to the inherent limitations of resolution and sensitivity of Capillary Electrophoresis, it is theoretically possible that some monoclonal gammopathies may not be detected by this analytical method. If the clinical case presentation is suggestive of a gammopathy, the electropherogram should be closely examined for any sign of abnormality. If in doubt, attempt to immunotype any abnormal gammaglobulins present by Immunodisplacement and/or agarose gel Immunofixation.

There are some rare incidences of unusual traces being encountered using serum protein analysis where traces are displayed as poorly resolved, with wide peaks and sometimes show faster or slower than normal migration times.

If these unusual trace symptoms are repeatable in successive runs, are not identified in other samples and cannot be attributed to system performance, the cause of these symptoms may be due to an euglobulin, cryoglobulin or "sticky" IgM monoclonol.

These samples should be checked for turbidity, diluted 1:100 in appropriate V8 sample diluent and repeated. If unresolved by the previous steps treat the sample with 5 µL of 2-mercaptoethanol (BME) to 1 mL of serum and incubate for 1 hour at 37°C and then repeat the run.

REFERENCE VALUES

SPE:

Using 42 normal specimens from male and female adult donors, the following normal ranges were obtained (these are presented as a guideline only):

Protein Fraction	Mean (%)	± 2SD Range
Albumin	61.0	54.4 - 67.6
Alpha-1	4.2	2.9 - 5.4
Alpha-2	9.4	6.6 - 12.2
Beta	11.8	9.7 - 14.6
Gamma	13.6	8.4 - 18.9

SPE (Fast CE):

Using 42 normal specimens from male and female adult donors, the following normal ranges were obtained (these are presented as a guideline only):

Protein Fraction	Mean (%)	± 2SD Range
Albumin	53.8	48.4 - 59.2
Alpha-1	6.6	4.9 - 8.2
Alpha-2	10.8	8.0 - 13.5
Beta	14.3	11.7 - 16.8
Gamma	14.6	9.5 - 19.6

PERFORMANCE CHARACTERISTICS

Helena Laboratories has determined the following performance characteristics according to CLSI EP05-A3. Samples were run twice per day with two repetitions per run for 20 days. Each laboratory should establish its own performance data.

Precision:

SPE:

Normal Control Fraction	Mean (%)	Within Run CV (%)	Between Run CV (%)	Between Day CV (%)	Total CV (%)
Albumin	66.0	0.9	0.4	0.0	1.0
Alpha-1	4.5	4.7	4.4	0.0	6.4
Alpha-2	9.7	3.4	3.3	3.0	5.6
Beta	9.8	2.5	2.7	1.9	4.0
Gamma	10.0	2.2	0.9	1.1	2.6

Abnormal Control Fraction	Mean (%)	Within Run CV (%)	Between Run CV (%)	Between Day CV (%)	Total CV (%)
Albumin	56.0	0.8	0.8	0.0	1.1
Alpha-1	4.1	3.2	3.3	0.8	4.6
Alpha-2	8.2	4.0	3.7	3.8	6.4
Beta	9.4	3.3	2.7	0.0	4.3
Gamma	22.4	1.6	1.5	0.0	2.1

SPE (Fast CE):

Normal Control Fraction	Mean (%)	Within Run CV (%)	Between Run CV (%)	Between Day CV (%)	Total CV (%)
Albumin	58.1	2.5	0.0	2.3	3.4
Alpha-1	7.3	10.7	0.0	6.8	12.7
Alpha-2	11.4	3.6	1.2	3.3	5.0
Beta	12.5	3.3	1.4	3.1	4.8
Gamma	10.8	3.1	1.1	1.8	3.7

Abnormal Control Fraction	Mean (%)	Within Run CV (%)	Between Run CV (%)	Between Day CV (%)	Total CV (%)
Albumin	49.6	1.8	0.3	1.8	2.6
Alpha-1	5.9	9.0	0.0	8.4	12.4
Alpha-2	10.1	4.3	0.6	3.7	5.7
Beta	12.1	3.3	0.0	2.5	4.2
Gamma	22.3	1.7	0.0	1.1	2.0

Sensitivity:

The V8 Serum Protein SPE Kit can detect serum proteins at levels of 0.21 g/L (21 mg/dL). The V8 Serum Protein SPE Kit (Fast CE) can detect serum proteins at levels of 0.25 g/L (25.0 mg/dL).

BIBLIOGRAPHY

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4. Tietz, N.W. (Ed.), Textbook of Clinical Chemistry, W.B. Saunders Co., Philadelphia, pages 579-582, 1986.
5. Ritzmann, S.E. (Ed.), Protein Abnormalities Volume 1 : Physiology of Immunoglobulins - Diagnostic and Clinical Aspects', Allen R. Liss, Inc., New York, 1982.

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