V8 Nexus Hemoglobin UltraScreen Procedure

Cat. No. 1828

INTENDED USE

The V8 Nexus Hemoglobin UltraScreen method is designed for the separation of normal hemoglobins (A, A_2, and F) in human blood samples, and for the detection of major hemoglobins variants (S and C) by using a capillary zone electrophoresis (CZE) buffer with the V8 instrument. The V8 Nexus Hemoglobin UltraScreen test is indicated for use in patients two (2) years of age and older. This test is designed for in-vitro diagnostic use only in conjunction with other laboratory and clinical findings.

The V8 instrument is an automated analyzer which performs a complete hemoglobin profile for quantitative analysis of the normal hemoglobin fractions A, A_2 and F and for the detection of major hemoglobin variants S and C. The assay is performed on the hemolysate of venous whole blood collected in tubes containing K_2EDTA as the anticoagulant.

For In Vitro Diagnostic use.

Rx Only

SUMMARY

Over 400 variant hemoglobins are now known, some of which may cause serious clinical effects, especially when present in the homozygous state, or in combination with another hemoglobin type. Wintrobe[1] divides the abnormalities of hemoglobin synthesis into 3 groups:

(1) Production of an abnormal protein molecule.

(2) Reduction in the amounts of normal protein synthesis.

(3) Developmental anomalies.

PRINCIPLE

The V8 instrument is a counter top electrophoresis system capable of separating variant and normal hemoglobins. Capillary electrophoresis is achieved using a microbore, fused silica capillary filled with an appropriate electrolyte medium under high voltage. Separation of the charged analytes is due to differential migration in an electrical field, whereby the charged particles migrate toward an electrode with an opposite charge. The application of the EOF (electro-osmotic flow) and Coulomb force allows all charged species to be detected at one end of the capillary within zones of migration, due to mass to charge ratio separation alongside EOF induced movement towards the optical detector.

With so many variant hemoglobins, there is no one technique which can unequivocally identify each hemoglobin variant. The capillary zone electrophoresis method available on the V8 system will separate the more common variants from the normal A peak. The run positions of variant hemoglobins can be used to give an initial identification of the variant. For a more positive identification another technique or protein sequencing must be used as per IFCC guidance.

REAGENTS

1. **V8 Hemoglobin UltraScreen Buffer (1 x 500 mL)**
   Ingredients: The buffer contains Taurine, L-Arginine, Tris, and Proclin 950.
   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 15-30°C and is stable until the expiration date indicated on the label. Opened bottles placed on the V8 instrument are stable for 5 months. **DO NOT FREEZE THE LIQUID.**
   Signs of Deterioration: The buffer should be a clear, colorless solution. Discard if solution shows extreme color change or cloudiness indicating microbial contamination.
2. **V8 Hemoglobin UltraScreen Diluent (2 x 20 mL)**
   Ingredients: The diluent contains Triton X-100 and Proclin 950.
   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 15-30°C and is stable until the expiration date indicated on the label. Opened bottles placed on the V8 instrument are stable for 5 months. **DO NOT FREEZE THE LIQUID.**
   Signs of Deterioration: The diluent should be a clear, colorless solution. Discard if solution shows extreme color change or cloudiness indicating microbial contamination.

INSTRUMENT

The Helena V8 Nexus capillary electrophoresis analyzer must be used for sample analysis. Refer to the Operator's Manual for detailed instructions.

SPECIMEN COLLECTION AND HANDLING

Specimen: Freshly collected K_2EDTA anticoagulated whole blood is the specimen of choice.

Specimen Storage and Stability: Samples can be stored at 2-8ºC for up to 7 days. However, best results are obtained with fresh samples as the formation of degradation product will complicate interpretation of the resulting hemoglobin trace.

Interfering Factors

Inaccurate results may be obtained on specimens left uncovered, due to evaporation. Samples older than 7 days cannot be used. Aged samples containing E variants can sometimes appear with a degradation peak to the right of the A_2 peak. This degradation peak may be marked as A_2 and therefore cause other peaks to be mislabeled in the trace. This can be corrected by manually locking peaks through the "edit peaks" right click menu. HbE is a hemoglobin variant with a similar electrophoretic mobility to HbA_2. As a result of this, HbE increases the A_2% in both heterozygous and homozygous expression. This interference is well documented and is present in HPLC and CZE methods. Interference should be taken into account when assessing a reference range for compound expression of beta thalassemia and HbE. As a result, a beta thalassemia cut off in the presence of HbE should be established and validated in the local population. HbA_2 may be elevated in the presence of HbS or HbC, Methemoglobin migrates in the position of HbF in older samples.

Common interfering factors including lipids and bilirubin were tested using CLSI EP07-A2 guidelines.[2] Lipid concentrations approaching 25 g/L and bilirubin concentrations approaching 25 mg/dL demonstrated no qualitative or quantitative interference on the performance of the UltraScreen assay.

USAGE MATRIX

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

40+ tests per session:	300 tests per bottle
32 tests per session:	272 tests per bottle
24 tests per session:	227 tests per bottle

PROCEDURE

Materials provided: The following materials needed for the procedure are contained in the V8 Hemoglobin UltraScreen Kit (Cat. No. 1828). Individual items are not available.

V8 Hemoglobin UltraScreen Buffer (1 x 500mL)
V8 Hemoglobin UltraScreen Diluent (2 x 20mL)
V8 Filter Unit (1)
V8 Sample Cups (3 pkg)

Materials provided but not contained in the above kit:

Items	Cat. No.
V8 Nexus CE Analyzer	1825
V8 Storage Buffer	1831
V8 Maintenance Buffer	1832
V8 Clinical Waste Drawer Inserts	1820
V8 AFSA2 Hemo Control	1812

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 that Storage Buffer, Maintenance Buffer, disposable cups and waste container are onboard and in their correct positions.
2. Switch on the V8 instrument, launch Platinum, and begin a new V8 session. In Platinum, ensure that **Hb UltraScreen** is selected as the default method or use test ordering to assign the Hb UltraScreen assay to specific samples.
3. If prompted by the V8 instrument, install the V8 Hemoglobin Ultrascreen 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 Hemoglobin UltraScreen 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 Hemoglobin UltraScreen 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 Hemoglobin UltraScreen 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. Invert primary collection tubes several times to mix the whole blood. Remove caps and load the sample tubes into the sample racks, ensuring that the barcodes are visible through the rack window.
9. Load sample racks onto the left-hand side of the V8 sample transport area and close the lid.
   NOTE: The analyzer should not have more than 2 racks onboard at a time due to the settling of red blood cells.
10. The V8 instrument will automatically commence analysis of all loaded samples, and the results will be transferred to Platinum.
11. After analysis, and if required, initiate V8 shut-down mode by switching off the instrument.
    NOTE: It is important that the V8 is post-conditioned correctly at the end of the day. If the buffer port is to be left empty after V8 Hemoglobin UltraScreen Buffer usage, it is recommended to purge with V8 Storage Buffer before post-condition to prevent the line from drying out.

QUALITY CONTROL

The V8 $AFSA_2$ Hemo Control (Cat. No. 1812) is to be used as a quantitative and/or qualitative control for the Hemoglobin UltraScreen assay on the V8 capillary electrophoresis system. Refer to the package insert provided with the control for the appropriate assay values and migration patterns.

INTERPRETATION OF RESULTS

Most hemoglobin variants cause no discernible clinical symptoms, so are of interest primarily to research scientists. Variants are clinically important when their presence leads to sickling disorders, thalassemia syndromes, lifelong cyanosis, hemolytic anemias, erythrocytosis, or if the heterozygote is of sufficient prevalence to warrant genetic counseling. The combinations of HbSS, HbSD-Los Angeles, and HbSO-Arab lead to serious sickling disorders.[3] Several variants including HbH, E-Fort Worth and Lepore cause a thalassemic blood picture.[3]

The two variant hemoglobins of greatest importance in terms of frequency and pathology are HbS and HbC.[3] Sickle cell anemia (HbSS) first manifests itself at about 5-6 months of age. The clinical course presents agonizing episodes of pain and temperature elevations with anemia, listlessness, lethargy and infarct in virtually all organs of the body. The individual with homozygous HbCC suffers mild hemolytic anemia which is attributed to the precipitation or crystallization of HbC within the erythrocytes. Cases of HbSC disease are characterized by hemolytic anemia that is milder than sickle-cell anemia.

The thalassemias are a group of hemoglobin disorders characterized by hypochromia and microcytosis due to the diminished synthesis of one globin chain (α or β) while synthesis of the other chain proceeds normally.[4,5] This unbalanced synthesis results in unstable globin chains. These precipitate within the red cell, forming inclusion bodies that shorten the life span of the cell. In α-thalassemias, the α-chains are diminished or absent, and in the β-thalassemia, the β-chains are affected. Another quantitative disorder of hemoglobin synthesis, hereditary persistent fetal hemoglobin (HPFH), represents a genetic failure of the mechanisms that turn off gamma chain synthesis at about four months after birth, which results in a continued high percentage of HbF. It is a more benign condition than the true thalassemias and persons homozygous for HPFH have normal development, are asymptomatic and have no anemia.[5]

The most common hemoglobin abnormalities**:**

Sickle Cell Trait

This is a heterozygous state showing HbA, HbS and a normal amount of HbA_2. Results show hemoglobins in the HbA and HbS migratory positions (zones).

Sickle Cell Anemia

This is a homozygous state showing almost exclusively HbS, although a small amount of HbF may also be present.

Sickle-C Disease

This is a heterozygous state demonstrating HbS and HbC.

Sickle Cell-Thalassemia Disease

This condition shows HbA, HbF, HbS and HbA_2.

In Sickle Cell β°-Thalassemia HbA is absent.

In Sickle Cell β+-Thalassemia HbA is present in reduced quantities.

Thalassemia-C Disease

This condition shows HbA, HbF and HbC.

C Disease

This is a homozygous state showing almost exclusively HbC.

Thalassemia Major

This condition shows HbA, HbF and HbA_2.

LIMITATIONS

1. The use of aged or frozen samples may produce additional artifact peaks caused by hemoglobin degradation products.
2. Globin chain analysis (both acid and alkaline) and structural studies may be necessary in order to positively identify some of the rarer hemoglobin variants.
3. It is recommended that the result is considered as part of the entire clinical picture.
4. Due to the resolution limits of capillary zone electrophoresis, it is possible that some hemoglobin variants may co-migrate with HbA or other variants using this method.
5. The V8 Hemoglobin UltraScreen kit has not been evaluated for use with dried blood spots (DBS) and the neonate subpopulation pediatric group.
6. A second test method is necessary for confirmation of abnormal hemoglobins detected.
7. Elevated HbA_2 results should be interpreted with caution in patients with abnormal hemoglobins.

REFERENCE VALUES

A reference range was established from 132 healthy individuals from 3 independent institutions using CLSI C28-A3 measurements.[6]

HbA: 96.6-97.9%

HbA_2: 2.1-3.4%

Note: the reference range for HbF can be below the reportable limit of the assay.

Helena Laboratories recommends each laboratory establish its own reference range.

PERFORMANCE CHARACTERISTICS

Precision/Reproducibility

Lot to Lot:

Precision studies were performed using one lot of $AFSA_2$ control, three lots of V8 Hemoglobin UltraScreen reagent kits, one V8 Nexus instrument and one operator. The tests were run 5 days x 2 runs/day x 8 reps/run. Each hemoglobin fraction has 240 total determinations (80 per fraction/lot). Within-run, between-run, between-day, between-lot and total precision were calculated. Acceptance precision criteria are all CVs for all Hemoglobin fractions ≤ 10%.

All hemoglobin fractions met acceptance criteria demonstrating acceptable lot to lot precision.

			Within-run		Between-run		Between-day		Between-lot		Total	
Fraction	N	Mean %	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
A	240	42.9	0.26	0.6	1.63	3.8	0.26	0.6	0.43	1.0	1.72	4.0
F	240	33.3	0.17	0.5	1.03	3.1	0.20	0.6	0.27	0.8	1.10	3.3
S	240	21.8	0.26	1.2	0.57	2.6	0.37	1.4	0.57	2.6	0.92	4.2
A_2	240	2.0	0.07	3.5	0.07	3.4	0.09	4.4	0.09	4.5	0.14	7.0

Instrument to Instrument:

Precision studies were performed using fresh and refrigerated venous K_2EDTA patient samples with known hemoglobin variants. Normal $HbAA_2$, HbAF (elevated F), $HbASA_2$ (elevated S and A_2) and $HbAA_2C$ (elevated C and A_2) were analyzed over the course of 5 days x 2 runs/day x 8 reps per run study. One V8 UltraScreen reagent kit lot, three V8 Nexus instruments and a single operator were employed. Each sample has 240 total determinations per hemoglobin fraction (80 per instrument). Within-run, between-run, between-day, between-instrument and total precision were calculated. Acceptance precision criteria for patient samples are as all CVs for all Hemoglobin fractions ≤ 10%.

All hemoglobin fractions met acceptance criteria demonstrating acceptable precision.

			Within-run		Between-run		Between-day		Between-Instrument		Total	
HbA Sample	N	Mean %	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
1	240	96.9	0.0	0.0	0.0	0.0	0.10	0.1	0.10	0.1	0.10	0.1
2	240	34.8	0.35	1.0	0.66	1.9	0.42	1.2	0.42	1.2	0.87	2.5
3	240	61.0	0.37	0.6	0.12	0.2	0.43	0.7	0.67	1.1	0.79	1.3
4	240	58.0	0.35	0.6	0.29	0.5	0.58	1.0	0.70	1.2	0.93	1.6

			Within-run		Between-run		Between-day		Between-Instrument		Total	
HbF Sample	N	Mean %	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
2	240	65.2	0.33	0.5	0.65	1.0	0.46	0.7	0.39	0.6	0.85	1.3

			Within-run		Between-run		Between-day		Between-Instrument		Total	
HbA_2 Sample	N	Mean %	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
1	240	3.1	0.05	1.5	0.00	0.1	0.05	1.6	0.05	1.6	0.07	2.2
3	240	3.6	0.05	1.3	0.01	0.4	0.05	1.3	0.05	1.3	0.07	1.9
4	240	5.5	0.07	1.2	0.06	1.1	0.07	1.2	0.07	1.2	0.12	2.1

			Within-run		Between-run		Between-day		Between-Instrument		Total	
HbS Sample	N	Mean %	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
3	240	34.5	0.35	1.0	0.17	0.5	0.38	1.1	0.59	1.7	0.76	2.2

			Within-run		Between-run		Between-day		Between-Instrument		Total	
HbC Sample	N	Mean %	SD	CV	SD	CV	SD	CV	SD	CV	SD	CV
4	240	36.5	0.37	1.0	0.29	0.8	0.58	1.6	0.73	2.0	0.95	2.6

LINEARITY / SENSITIVITY

The linearity of the V8 Nexus UltraScreen procedure was evaluated following CLSI EP06-A guidelines.[7] The studies were performed using the a single V8 Nexus Hemoglobin UltraScreen reagent kit, a single V8 Nexus and venous K_2EDTA samples containing different levels of each hemoglobin fraction (9 levels for each fraction). The tests were determined to be linear over the following ranges: HbA: 3.7-97.2%, HbF: 1.1-68.7%, HbS: 5.8-78.8%, HbA_2: 1.7-7.6%, HbC: 1.4-42.6%.

CORRELATION STUDIES

Clinical correlation studies were performed at three separate institutions. HbA, HbA_2, HbF, HbS, and HbC fractions were analyzed with the V8 Nexus Hemoglobin UltraScreen method versus the predicate capillary electrophoresis method. The degree of agreement with the predicate is outlined below.

Fraction	N	Range	R	Slope	95% CI	Intercept	95% CI
HbA	320	6.8-97.2	0.999	0.990	0.984,0.998	0.78	0.12,1.40
HbA_2	412	1.6-6.9	0.957	1.000	1.000,1.000	0.05	0.05,0.05
HbF	175	1.1-68.0	0.993	1.000	0.985,1.020	0.70	0.52,0.73
HbS	143	9.6-78.6	0.994	0.929	0.909,0.948	2.40	1.67,3.22
HbC	33	23.6-42.3	0.975	1.000	0.925,1.103	0.60	-2.70,3.06

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