No special patient preparation is

**Patient Preparation:**

EDTA as an anticoagulant is the specimen of choice. Heparin or citrate tubes may also be used if desired. However, fluoride and oxalate should not be used.

Fresh whole blood collected in tubes containing

**Specimen:**

WARNING: FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT PIPETTE ANY COLUMN SUPERNATANT BY MOUTH.

**Ingredients:**

The reagent should be a clear, colorless solution. Signs of Deterioration:

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Helena Laboratories wants to provide to its customers what it has come to be known as the "Helena advantage." Helena's Laboratory Products are distributed on an exclusive basis and are regarded as sufficient evidence for the diagnosis of

**PRINCIPLE:**

The Helena Beta-Thal HbA2 Quik Column® Procedure is an anion exchange chromatography methodology for the quantitation of HbA2.

**SUMMARY:**

The accurate quantitation of hemoglobin A2 (HbA2) in the clinical laboratory is essential for the differential diagnosis of several anemias and the thalassemias. Elevated HbA2 is widely regarded as sufficient evidence for the diagnosis of β-thalassemia trait. However, it may be normal if iron deficiency co-exists with the β-thalassemia trait. In the laboratory confirmation of the diagnosis of β-thalassemia trait, HbA2 levels should be considered in conjunction with family history and laboratory data including serum iron and iron binding capacity, red cell morphology, hemoglobin, hematocrit, and mean corpuscular volume (MCV). Total HbA2 levels expressed in mg/dl of whole blood in conjunction with the MCV have been reported as criteria for the differential diagnosis of β-thalassemia minor, iron deficiency anemia, and other hypochromic microcytic anemias.

HbA2 has been quantitated using cellulose acetate electrophoresis followed by elution or densitometry. Anion exchange chromatography, and immunochromatographic methods. Cellulose acetate electrophoresis followed by densitometry is an excellent screening method but greater accuracy is required for quantitating HbA2.

**Preparation for Use:**

Each Kit Contains:

- HbA2 Developer (1 x 130 mL)
- HbA2 Developer (1 x 30 mL)
- Quik Column Rack (1)
- Quik Column Collection Tubes (10 small tubes, 10 large tubes)
- Normal HbA2, Quik Column Control (5 x 1.0 mL)
- Abnormal HbA2, Quik Column Control (5 x 1.0 mL)
- HbA2, Quik Column® Equipment and Supplies

**Before storing the columns for use, the following instructions must be followed:**

1. **Storage and Stability:** The columns should be stored at 2 to 8°C and are stable until the expiration date indicated on the box. Allow columns to warm to room temperature before use.

2. **WARNING:** FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT PIPETTE BY MOUTH.

**Preparation for Use:** The reagent is ready for use as packed.

**Storage and Stability:** The reagent should be stored at 2 to 8°C and is stable until the expiration date indicated on the bottle.

**Signs of Deterioration:** The reagent should be clear, colorless solution.

**3. HEMOLYSATE REAGENT-C**

**Ingredients:** Hemolysate Reagent-C is deionized water with 0.1% Triton X-100 and preservatives added.

**Storage and Stability:** The reagent should be stored at 2 to 8°C and is stable until the expiration date indicated on the bottle.

**Signs of Deterioration:** The reagent should be a clear, colorless solution.

**INSTRUMENTS**

A spectrophotometer capable of reading absorbance accurately at 415 nm with a range of 0.0-2.0 absorbance (Abs) must be used. The HemeSpec® Plus (Cat. No. 1103) is recommended.

**SPECIMEN COLLECTION AND HANDLING**

Specimen: Fresh whole blood collected in tubes containing EDTA as an anticoagulant is the specimen of choice. Heparin or citrate tubes may also be used if desired. However, fluoride and oxalate should not be used.

**Patient Preparation:** No special patient preparation is necessary.
INTERPRETATION OF RESULTS

Columns should establish their own normal ranges. The reference range of HbA2 percentages using the Beta-Thal HbA2 Quik Column methodology for normal adults has been determined. Fifty normal adults 18 years of age or older were assayed by 3 different technicians running 50 columns each. The HbA2 expected range determined from the study is as follows:

Normal HbA2 Range = 2.2 - 3.3%

All laboratories using the Helena Beta-Thal HbA2 Quik Column should establish their own normal ranges.

INTERPRETATION CONSIDERATIONS

Reported HbA2 percentages in normal individuals vary according to the procedure employed. Values of 1.5-3.5% have been reported. Urinary HbA2 measurement is not considered normal. No exact boundaries exist between normal and abnormal values. Results of HbA2 assays must be interpreted in conjunction with patient history, total hemoglobin values and other clinical and laboratory findings. Any value between 3.5% and 8.0% is considered indicative of β-thalassemia trait. Values above 8% indicate the presence of additional hemoglobinopathies such as HBC, E, D, G, S or S-G Hybrid which elute with HbA2.

Table 1: Percentages of the HbA2 in some hematological disorders

<table>
<thead>
<tr>
<th>Disorder</th>
<th>No. Mean HbA2</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iron deficiency anemia</td>
<td>6</td>
<td>1.5</td>
</tr>
<tr>
<td>Polycythemia vera</td>
<td>3</td>
<td>2.3</td>
</tr>
</tbody>
</table>

The columns and all reagents should be equilibrated to room temperature (21 to 23°C) before running the procedure. Keep unused columns at 2 to 8°C.

PERSISTENT HIGH HbF TRAIT

HbF values have a tendency to rise in some hematological disorders. Values exceeding 5% of the total hemoglobin are reported.

The columns and all reagents should be equilibrated to room temperature (21 to 23°C) before running the procedure. Keep unused columns at 2 to 8°C.

The results of the Beta-Thal HbA2 Quik Column Method may be affected by the following conditions:

1. Incorrect preparation of the column
   a. Failure to completely resuspend the contents of the column may cause slow flow and erroneous results. Time must be allowed after resuspension for the formation of a distinct interface between the resin and supernatant. Any trapped bubbles may be removed with a Pasteur pipette.
   b. The bottom tip closure must be removed immediately after resuspension. Resuspension must be repeated when the column is allowed to sit with the bottom closure in place after resuspension. Failure to do so may cause resealed supernatant to re-enter the column.
   c. As soon as the resin re-wards, the remaining super-natant must be aspirated and discarded.

2. Inconsistent development
   d. To avoid back pressure in the column, do not remove the bottom tip closure before removing the top cap closure of the column. A 10 mL large bore syringe may slow or stop the flow rate, leading to erroneous results.

3. Molecular and reagents
   e. The columns and all reagents should be equilibrated to room temperature (21 to 23°C) before running the procedure. Keep unused columns at 2 to 8°C.

4. Disturbance of the resin during the procedure may cause erroneous results.

5. Resin drying out
   f. It is important not to allow the top of the resin to dry out before adding developer. No more than 5 minutes should elapse from the time the column stops flowing (during preparation for use) until the Developer is added.

6. Exposure of the column to extreme conditions
   g. The columns must not be exposed to direct sunlight or excessive heat (>30°C) or cold (<21°C) during the performance of the test. The column must not be frozen at any time.

7. Abnormal Hemoglobins
   h. Some of the abnormal hemoglobins (HbS,C,E,D,G,S-Hybrid) are eluted with HbA2 in this methodology. The presence of the abnormal hemoglobins should be confirmed by electrophenotypic techniques. HbF does not interfere with HbA2 quantitation.
   i. The investigator should be suspicious of the HbA2 assay, as HbA2 is often normal in hemoglobin Aß thalassemia. If necessary, specimens may be stored up to 14 days at 2 to 8°C.
   j. Persistent high HbF trait may persist for up to 3 hours at 2°C.

8. Incomplete Sample Absorption
   k. Failure to complete the resuspension of the sample into the resin before addition of the developer can be detected by a reddish tinge to the developer in the column.

9. Linearity of the Spectrophotometer
   l. A broad linear range is obtained on the Beta-Thal HbA2 Quik Column.

Use of a Spectrophotometer with inadequate linearity between 1.0 D.O. and 2.0 D.O. produces high values.

TROUBLESHOOTING

Presented below is a troubleshooting guide for the Beta-Thal HbA2 Quik Column Method. At the end of each symptom is a set of numbers in parentheses. These numbers refer to the specific sections of the Troubleshooting guidelines to which the symptom applies.

1) Excessive time is required for the hemolysate to absorb into the resin (>5 minutes), or for Developer to flow through the column (20-30 minutes to 2 hours).
2) Inconsistent development. Time required is not equal to the time specified in the test procedure.
3) Measured HbA2 concentration is lower than expected (values <1.5%).
4) Opaque or cloudy supernatant.
5) Hemolysate will have a glossy appearance when viewed from above until the sample is completely absorbed by the resin. Upon complete absorption, the top of the resin will have a dull, mat-like appearance.
6) Following the absorption of the sample into the resin bed, slightly apply 2.5 mL of HbA2 Developer to the column. Excessive force when applying the developer may disturb the resin bed and cause erroneous results.
7) Resin drying out.
8) Failure to allow complete absorption of the sample into the resin before addition of the developer can be detected by a reddish tinge to the developer in the column.
9) Exposure of the column to extreme conditions.
10) Resin drying out.
11) Exposure of the column to extreme conditions.
12) Inconsistent development.
13) Persistent high HbF trait may persist for up to 3 hours at 2°C.
14) Linearity of the Spectrophotometer.
15) Use of a Spectrophotometer with inadequate linearity between 1.0 D.O. and 2.0 D.O. produces high values.

BIBLIOGRAPHY

Interfering Substances: See LIMITATIONS for a complete discussion of interfering substances and other limiting factors.

Specimen Storage: The use of fresh blood samples is recommended. If necessary, specimens may be stored up to 14 days at 1°C.

Specimen Preparation: Detailed instructions for specimen preparation are included in the STEP-BY-STEP METHOD.

PROCEDURE

Materials Provided:
The following materials are provided in the Beta-Thal HbA2 Quik Column Kit (Cat. No. 5341).
- Beta-Thal HbA2 Quik Column
- Hemolysate

STEP-BY-STEP METHOD

For each patient question to be performed: Obtain a 1 Quik Column
- Add 250 µL of Hemolysate Reagent-C to the test tube. (approximately 30 minutes to 2 hours). The eluate contains the HbA2. See LIMITATIONS for abnormal hemoglobins which may also elute with the HbA2.

1. Place the small collection tube (3 mL) at room temperature.
2. Add 250 µL of HbA2 Developer to the column. (approximately 30 minutes to 2 hours)
3. Gently shake the tube to achieve complete absorption of the sample. Complete lysis of the sample is essential for accurate results. If, after 5 minutes, the sample is not completely lysed, the freeze-thaw technique may be used to lyse samples.
4. Allow the sample to stand at least 5 minutes prior to use.

b. Packed Cells

Alternatively, saline-washed packed red blood cells may be used in the Quik Column (large). To 25 µL of packed washed cells, add 300 µL of Hemolyse Reagent-C. Shake vigorously and allow to stand for 5 minutes.

4. Prepare the columns for use as follows:

a. Let the resin re-pack to the scribed line with deionized water. The volume of the column should be 15 mL.

b. Place 100 µL of Hemolysate Reagent-C to the test tube. (approximately 30 minutes to 2 hours). The eluate contains the HbA2. See LIMITATIONS for abnormal hemoglobins which may also elute with the HbA2.

5. Place the small collection tube (3 mL) at room temperature.

Quality Control:
The Normal HbA2 Quik Column Control (Cat. No. 5339) and Abnormal HbA2 Quik Column Control (Cat. No. 5333) are available from Helena Laboratories. Controls should be run with each set of unknowns for continued quality control. The low normal hemoglobin for the HbA2 has been assayed for percentage. The controls must be reconstituted as directed in the package insert. No further dilution is necessary before application to a Quik Column.

RESULTS

Calculation of Unknown: HbA2 may be determined using a standard spectrophotometer and performing calculations according to the formula:

\[ \text{HbA2%} = \frac{\text{A} \times 100}{\text{HbA2%}} \]

where:
- A = absorbance of the contents of the small collection tube at 415 nm (HbA2 fraction).
- HbA2% = percentage of HbA2 in the sample.

Examples:
1. Sample yielding absorbance values of 0.016 for the small tube and 0.274 for the large tube is 2.7% HbA2, or 2.7% of total hemoglobin (Hb).
2. The method is also suitable for an HbA2 percent of 2.0% using the above formula to perform the calculation.

REFERENCE RANGE

The reference range of HbA2, percentages using the Beta-Thal HbA2 Quik Column methodology for normal adults has been determined. Fifty normal adults 18 years of age or older were tested by 3 different technicians running 50 columns each. The HbA2, expected range determined from the study is as follows:

Normal HbA2 Range = 2.2 - 3.3 %

All laboratories using the Helena-Beta-Thal HbA2 Quik Column should establish their own normal ranges.

INTERPRETATION OF RESULTS

Reported HbA2 percentages in normal individuals vary according to the procedure employed. Values of 1.5-3.5% have been reported. Unless normal ranges are established, no exact boundaries exist between normal and abnormal values. Results of HbA2 assays must be interpreted in conjunction with patient history, total hemoglobin values and other clinical and laboratory findings. Any value between 3.5% and 8.0% is considered indicative of β-thalassemia trait. Values above 8% indicate the presence of additional hemoglobinopathies, HbC, E, D, G, S or S-G hybrid which elute with HbA2.

Table 1:

<table>
<thead>
<tr>
<th>Disorder</th>
<th>Donors</th>
<th>Mean HbA2%</th>
<th>Range HbA2%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy</td>
<td>21</td>
<td>2.9</td>
<td>2.0-3.9</td>
</tr>
<tr>
<td>Thalassemia trait</td>
<td>2</td>
<td>2.8</td>
<td>2.0-3.5</td>
</tr>
<tr>
<td>Hemoglobin S trait</td>
<td>2</td>
<td>2.5</td>
<td>1.9-3.0</td>
</tr>
<tr>
<td>Hemoglobin C trait</td>
<td>2</td>
<td>2.4</td>
<td>2.0-2.8</td>
</tr>
<tr>
<td>Iron deficiency anemia</td>
<td>2</td>
<td>2.3</td>
<td>1.8-2.8</td>
</tr>
<tr>
<td>Polycythemia vera</td>
<td>2</td>
<td>2.3</td>
<td>2.0-2.5</td>
</tr>
<tr>
<td>β-Thalassemia trait</td>
<td>2</td>
<td>2.2</td>
<td>1.8-2.6</td>
</tr>
</tbody>
</table>

LIMITATIONS

The results of the Beta-Thal HbA2 Quik Column Method may be affected by the following conditions:

1. Incorrect preparation of the column:
   a. Failure to completely resuspend the contents of the column may cause slow flow and erroneous results. Time must be allowed after resuspension for the formation of a distinct interface between the resin and supernatant. Any trapped bubbles may be removed with a Pasteur pipette.
   b. The bottom tip closure must be removed immediately after resuspension. Resuspension must be repeated if the column is allowed to sit with the bottom closure in place after resuspension. Failure to do so may cause results to be delayed.
   c. As soon as the resin re- packs, the remaining supernatant must be aspirated and discarded.

d. To avoid back pressure in the column, do not remove the bottom tip closure before removing the top cap closure. If the column is frozen, do not thaw the resin may slow or stop the flow rate, leading to erroneous results.

2. Incorrect developer flow:
   Should the developer cease to flow through the column during the procedure, the column must be discarded and the procedure repeated with a fresh column.

3. Temperature of column and reagents
   The columns and all reagents should be equilibrated to room temperature (21 to 30°C) before running the procedure. Keep unused columns at 2°C to 8°C.

4. Disturbance of resin
   Any disturbance of the resin during the procedure may cause erroneous results.

5. Resin drying out:
   It is important not to allow the top of the resin to dry out before adding developer. No more than 5 minutes should elapse from the time the column stops flowing (during preparation for use) until the Developer is added.

6. Exposure of the column to extreme conditions
   The columns must not be exposed to direct sunlight or extreme heat (> 30°C) or cold (< 21°C) during the performance of the test. The column must not be frozen at any time.

7. Abnormal Hemoglobins
   a. Some of the abnormal hemoglobins (HbS,C,E,O,D,β-thalassemia trait) are eluted with HbA2 in this methodology. The presence of the abnormal hemoglobins should be confirmed by electrophoretic techniques. HbF does not interfere with HbA2 quantitation.
   b. The investigator should be suspicious of the HbA2 as falsely high. Although, Hemoglobin A band does not remain tight. This may occur if HbS is present in the sample. The resulting HbA2 values have a tendency to run higher than actual values.
   c. As the resin repacks, you will see an interface (with a distinct interface between the resin and supernatant). The presence of the abnormal hemoglobins should be confirmed by electrophoretic techniques. HbF does not interfere with HbA2 quantitation.

8. Incomplete Sample Absorption
   Failure to allow complete absorption of the sample into the resin before addition of the developer can be detected by a reddish tinge to the developer column.

9. Linearity of the Spectrophotometer
   Use of a Spectrophotometer with inadequate linearity between 1.0 O.D. and 2.0 O.D. produces high values.

10. Linearity of the Spectrophotometer
   Use of a Spectrophotometer with inadequate linearity between 1.0 O.D. and 2.0 O.D. produces high values.

TROUBLESHOOTING

Presented below is a troubleshooting guide for the Beta-Thal HbA2 Quik Column Method. At the end of each symptom is a set of numbers in parentheses. These numbers reference more complete explanations found in the LIMITATIONS Section of this procedure.

1) Excessive time is required for the hemoglobin to absorb into the resin (> 5 minutes), or for Developer to flow through the column (> 30 minutes to 2 hours).

2) Incorrect developer flow.

3) Abnormally high values are obtained. (7)

4) Use of cold developer produces low values. (3)

5) Adding developer to the columns too fast produces low values. (4,8)

BIBLIOGRAPHY

HbA2 Quik Column® Procedure

The Helena Beta-Thal HbA2 Quik Column® Procedure is a microchromatographic methodology for the quantitation of HbA2.

**SUMMARY**

The accurate quantitation of hemoglobin A2 (HbA2) in the clinical laboratory is essential for the differential diagnosis of several anemias and the thalassemias. Elevated HbA2 is widely regarded as sufficient evidence for the diagnosis of β-thalassemia trait. However, it may be normal if iron deficiency co-exists with the β-thalassemia trait. In the laboratory confirmation of the diagnosis of β-thalassemia trait, HbA2 levels should be considered in conjunction with family history and laboratory data including serum iron and iron binding capacity, cell morphology, red cell membrane, and mean corpuscular volume (MCV). The total HbA2 levels expressed in mg/dL of whole blood in conjunction with the MCV have been reported as criteria for the differential diagnosis of β-thalassemia minor, iron deficiency anemia, and other hypochromic microcytic anemias. HbA2 has been quantitated using cellulose acetate electrophoresis followed by elution or densitometry, anion exchange chromatography, and immunological methods. The anion exchange resin is a preparation of cellulose covalently coupled to small negatively charged cellulose. Electrophoresis of the anion exchange chromatography, 4-7 and immunochemical methods. 8, 9

**METHODS**

1. **Beta-Thal HbA2, Quik Columns**

   Each Kit Contains:
   - Beta-Thal HbA2, Quik Columns (50)
   - HbA2 Developer (1 x 130 mL)
   - Hemolysate Reagent-C (1 x 20 mL)

2. **Sickle-Thal HbA2 Quik Column Kit**

   Each Kit Contains:
   - Sickle-Thal HbA2 Quik Columns (25)
   - HbS Developer (1 x 300 mL)
   - Hemolysate Reagent-C (1 x 20 mL)

**PREPARATION FOR USE**

For purchase, see the form for this reagent, always flush the sink with copious quantities of water. This will prevent the formation of metallic fumes which, when highly concentrated, may be explosive. In addition to purging pipes with water, plumbing should occasionally be decontaminated with 10% NaOH.

**PREPARATION FOR USE**

For purchase, see the form for this reagent, always flush the sink with copious quantities of water. This will prevent the formation of metallic fumes which, when highly concentrated, may be explosive. In addition to purging pipes with water, plumbing should occasionally be decontaminated with 10% NaOH.

**STORAGE AND STABILITY**

The columns should be stored at 2 to 8°C and are stable until the expiration date indicated on the box. Allow columns to warm to room temperature before use.

**SIGNS OF DEGRADATION**

The column should contain a slightly yellowish grey-tone resin with a clear supernatant. A vivid yellow or yellow-green color may indicate bacterial contamination.

**REAGENTS**

1. **Beta-Thal HbA2, QUIC COLUMNS**

   **Ingredients:** Each column contains 300 mg of DEAE cellulose in 0.2 M glycine buffer with 0.01% potassium cyanide and 0.1% sodium azide.

   **WARNING:** FOR IN-VITRO DIAGNOSTIC USE ONLY. DO NOT PIPETTE BY MOUTH.

   This product contains Sodium Azide. To prevent the formation of toxic vapors, this reagent should not be mixed with acidic solutions. When discarding this reagent, always flush the sink with copious quantities of water. This will prevent the formation of metallic azides which, when highly concentrated, may be explosive.