AEROSET® & ARCHITECT® c8000®
OEM Reagent Training

MULTIGENT® Microalbumin

December 2004
Agenda

- Basic Information
- Sample Handling
- Expected Values
- Reagent Handling
- Reagent Configuration
- Calibration
- Quality Control
- Reaction Methodology
- Interfering Substances
- Precision
- Method Comparison
- Assay Specific Information
- Troubleshooting Tips
- Questions & Answers
Basic Information
Assays in Development for OEM launch

<table>
<thead>
<tr>
<th>Assay</th>
<th>Target launch date</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microalbumin (Wako)</td>
<td>January 2005</td>
</tr>
</tbody>
</table>
MULTIGENT® Microalbumin

- Reagent packaging will say:
  - Manufactured by Wako
  - Distributed by Abbott Laboratories

- Package insert contains AEROSET®/c8000® Assay configuration parameters

- Abbott list number on labeling

- Abbott Laboratories Customer Support phone number on package insert

- Reagent cross-contamination, high sample carryover, and required flagging were evaluated

- Assay was developed with technical input from Abbott
**Complaint Handling**

- Troubleshoot as for an Abbott assay
- Evaluate complaints for pRE (potential reportable event)
- Elevate as with Abbott assay when a Level II investigation is required
- Quality will transfer complaint to the vendor for investigation
- Vendor expected to complete complaint investigation within 30 days
Clinical Implications

- Microalbuminuria is a condition characterized by increased urinary excretion of albumin and can be used to predict diabetic nephropathy.
- Monitoring urinary microalbumin is an important component of treatment for both Type I & II diabetes mellitus.
- Early detection of glomerular damage, when minimal and reversible, is extremely important.
- Exercise within 24 hours, infection, fever, congestive heart failure, marked hyperglycemia, and marked hypertension may elevate urinary albumin excretion over baseline values.
Sample Handling
Sample Handling

Urine is the acceptable specimen

Methods of monitoring microalbumin in urine include:

- 24-hour urine collection
- Spot collection - requires analysis of both microalbumin and urine creatinine
Sample Handling

Acceptable preservatives:

- 6N Hydrochloric acid
- Acetic acid
- Chloroform
- Formalin
- Toluene
- Xylene
Sample Handling

Specimen storage:
- Analyze fresh specimens if possible
- Storage guidelines:
  - 24-hour collections
    - 2 weeks at 4°C
    - 5 months at –70°C
  - Spot samples
    - 6 days at 4°C
- Avoid repeated freeze/thaw cycles
- Adequately mix and centrifuge frozen specimens prior to testing to avoid reduced values
Expected Values
Expected Values

Spot Collection:

\[ \text{uAlb mg/L} \div \text{Crea-U mmol/L} = \text{mg/mmol} \]

OR

\[ (\text{uAlb } \mu g/mL \div \text{Crea-U mg/dL}) \times \frac{100 \text{ mL}}{1 \text{ dL}} = \mu g/mg \]

NOTE: The ratio of albumin to creatinine in a spot urine sample corrects for variations in hydration and avoids the sources of error associated with 24-hour and timed urine collections.

24-hour Collection:

\[ (\text{uAlb } \mu g/mL \times \text{Volume mL}) \div \frac{1000 \mu g}{1 \text{ mg}} = \text{mg/24 hr} \]

Timed Collection:

\[ (\text{uAlb } \mu g/mL \times \text{Volume mL}) \div \text{Time (min)} = \mu g/min \]
Medical Decision Levels per American Diabetes Association (ADA)

<table>
<thead>
<tr>
<th></th>
<th>Spot collection Microalbumin:Creatinine ratio</th>
<th>24-hour Collection (mg/24 hr)</th>
<th>Timed Collection (µg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µg/mg or mg/g (mg/mmol)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>&lt; 30</td>
<td>&lt; 2.5</td>
<td>&lt; 3.5</td>
</tr>
<tr>
<td>Microalbuminuria</td>
<td>30-299</td>
<td>2.5-29</td>
<td>3.5-29</td>
</tr>
<tr>
<td>Macro (clinical) Albuminuria</td>
<td>≥ 300</td>
<td>&gt; 30</td>
<td>&gt; 30</td>
</tr>
</tbody>
</table>
**Expected Values**

- Due to variability in urinary albumin excretion, at least two of three test results measured within a 6-month period should show elevated levels before a patient is designated as having microalbuminuria.

- Exercise within 24 hours, infection, fever, congestive heart failure, marked hyperglycemia, and marked hypertension may elevate urinary albumin excretion over baseline values.
Reagent Handling
Reagent Handling

- Reagent list number
  2K98-20

- Reagent kit configuration
  - Reagent 1: 2 x 53 mL in 55 mL cartridge
  - Reagent 2: 2 x 12 mL in 20 mL cartridge

- 500 tests per kit
Reagent Handling

- Reagent is liquid, ready-to-use, two reagent kit.
- Unopened reagents are stable until the expiration date when stored at 2 to 10°C.
- Do not freeze reagents.
- Reagents should be mixed thoroughly before use.
- Avoid the formation of foam.
- Do not mix materials from different kit lot numbers.
Reagent Handling

- **Storage 2 to 10ºC**
  - Kit stable to expiration date
  - Do not freeze or expose reagent to temperatures above 32ºC.

- **Reagent onboard stability**
  - 28 days (672 hours)
MULTIGENT® Microalbumin Configuration
**Assay Configuration**

- MULTIGENT® Microalbumin assay is manually configured
  Use assay parameters from the package insert.

- Reagent name is case sensitive: *c8000® Microalbumin reagent name: UALBO*
ARCHITECT® c8000® Assay Configuration

Configuring parameters for MULTIGENT® assays include performing the following procedures in order:

1. **Configure the calibrator.**
   Refer to Configure a calibrator in the ARCHITECT Systems Operations Manual.

2. **Configure the bar coded MULTIGENT Microalbumin reagent.**

3. **Configure the assay parameters.**
   Refer to Configure a photometric assay in the ARCHITECT System Operations Manual.
AEROSET® Assay Configuration

1. Load the reagents and perform a Reagent Scan.
   Refer to Reagent Configuration in Section 2, Installation Procedures and Special Requirements of the AEROSET System Operations Manual.

2. Configure the Microalbumin calibrator and control names and targets.
   Refer to Calibrator/Control Configuration in Section 2, Installation Procedures and Special Requirements of the AEROSET System Operations Manual.

3. Manually configure the assay using the parameters found in the package insert.
   Refer to Assay Configuration in Section 2, Installation Procedures and Special Requirements of the AEROSET System Operations Manual.
AEROSET® Reagent Carryover

MULTIGENT® Microalbumin must be line separated from:
- MULTIGENT Digoxin – LN 1E06-20
- Abbott Direct Bilirubin – LN 8G63-20

Reagent Probe SmartWashes are required if these assays are on the same line as MULTIGENT Microalbumin:
- Iron (IRON061, IRON052)
- ICT Diluent (ICTD061)
ARCHITECT® c8000® Reagent Carryover

To avoid reagent carryover between MULTIGENT® Digoxin and MULTIGENT Microalbumin:

Configure a Reagent Probe SmartWash™ using Detergent A for both R1 and R2 probes.
Calibration
Calibration

- Calibrators are liquid ready-to-use
- Calibrator list number
  2K98-02
- Calibrator kit configuration
  - 5 Calibrator levels
  - 1 x 2 mL bottles
Calibration

Calibrator stability
- Unopened calibrators are stable until expiration date on label when stored at 2 to 10°C
- Opened calibrators stable 6 months stored at 2 to 10°C
- Do not freeze, improper storage can affect assay performance

Calibrators are clear, colorless, odorless solutions
- Suspect deterioration if visible signs of precipitant, leakage, turbidity, microbial growth, or if calibration does not meet package insert claims or instrument-specific operations manual criteria.
Calibration Curves - AEROSET®
Calibration Curves - ARCHITECT® c8000®

Calibration curve...

Assay: uALB
Assay number: 2333
Reagent lot: AL859
Reagent S/N: 12366
Expiration date: 07.31.2005

Calibration status: Active
Cal date / time: 09.24.2003 / 17:11
Cal method: Spline
Calibration type: Full
Calibrator lot: 
Expiration date: 
Module / Serial No.: 1/c857005
Operator ID: FSE
Error code: 

<table>
<thead>
<tr>
<th>CAL_ID</th>
<th>CONC ug/mL</th>
<th>CAL ABSORBANCE</th>
<th>CAL FACTOR</th>
<th>REP1</th>
<th>REP2</th>
<th>REP3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blank</td>
<td>0.00</td>
<td>0.0459</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cal 1</td>
<td>5.00</td>
<td>0.0165</td>
<td>302.7755</td>
<td>0.0461</td>
<td>0.0459</td>
<td>0.0456</td>
</tr>
<tr>
<td>Cal 2</td>
<td>25.00</td>
<td>0.0700</td>
<td>373.9288</td>
<td>0.0623</td>
<td>0.0624</td>
<td>0.0626</td>
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<tr>
<td>Cal 3</td>
<td>100.00</td>
<td>0.2175</td>
<td>508.6279</td>
<td>0.1159</td>
<td>0.1156</td>
<td>0.1165</td>
</tr>
<tr>
<td>Cal 4</td>
<td>300.00</td>
<td>0.4965</td>
<td>716.7317</td>
<td>0.3617</td>
<td>0.2633</td>
<td>0.2644</td>
</tr>
<tr>
<td>Cal 5</td>
<td>500.00</td>
<td>0.7192</td>
<td>897.9011</td>
<td>0.7744</td>
<td>0.7651</td>
<td>0.7559</td>
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</tbody>
</table>

Abs

[Graph showing calibration curve with Abs on the y-axis and Concentration on the x-axis]

Curve expiration date / time:
Full: 11.04.2003 / 17:11
Adjust: 

30-3464/R1—December 2004
Quality Control
Quality Control

- Controls are liquid ready-to-use
- Control list number
  2K98-10
- Control kit configuration
  - 2 Control levels
  - 2 x 2 mL bottles
Quality Control

Control stability
- Unopened controls are stable until expiration date on label stored at 2 to 10°C. Do not use past expiration date.
- Opened controls are stable 6 months if stored at 2 to 10°C in original containers and kept free of contamination

Do not freeze controls

Improper storage of controls can affect assay performance
Reaction Methodology
**Reaction Methodology**

Microalbumin (sample) + Good’s buffer (R1) + TRIS buffer (R2) + Anti-human albumin (goat) antibody (R2)

Turbidity due to Microalbumin:Anti-human albumin (goat) Antibody complex (Insoluble)
Interfering Substances
Interfering Substances

Interference is $\leq 10\%$ for urine pH levels between 3 and 10.
Interfering Substances

Interference is $\leq 10\%$ for the compounds listed below, when used in the amounts shown in the package insert.

<table>
<thead>
<tr>
<th>Interferents</th>
<th>Interferent Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conventional Units</td>
</tr>
<tr>
<td>Ascorbic acid</td>
<td>400 mg/dL</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>25 mg/dL</td>
</tr>
<tr>
<td>Calcium</td>
<td>400 mg/dL</td>
</tr>
<tr>
<td>Creatinine</td>
<td>400 mg/dL</td>
</tr>
<tr>
<td>Glucose</td>
<td>4,000 mg/dL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>500 mg/dL</td>
</tr>
<tr>
<td>Hippuric acid</td>
<td>400 mg/dL</td>
</tr>
</tbody>
</table>
## Interfering Substances (continued)

<table>
<thead>
<tr>
<th>Interferents</th>
<th>Interferent Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conventional Units</td>
</tr>
<tr>
<td>Inorganic phosphorus</td>
<td>400 mg/dL</td>
</tr>
<tr>
<td>Potassium chloride</td>
<td>1,000 mg/dL</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>2,000 mg/dL</td>
</tr>
<tr>
<td>Urea nitrogen</td>
<td>400 mg/dL</td>
</tr>
<tr>
<td>Uric acid</td>
<td>100 mg/dL</td>
</tr>
<tr>
<td>Urobilinogen</td>
<td>20 mg/dL</td>
</tr>
</tbody>
</table>
**Interfering Substances**

Interference is ≤10% for the urine preservatives listed below when used in the amounts shown in the package insert.

<table>
<thead>
<tr>
<th>Urine Preservative</th>
<th>Preservative Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Conventional Units</td>
</tr>
<tr>
<td>6N Hydrochloric acid</td>
<td>4 mL/dL</td>
</tr>
<tr>
<td>Acetic Acid</td>
<td>1000 µL/dL</td>
</tr>
<tr>
<td>Chloroform</td>
<td>1000 µL/dL</td>
</tr>
<tr>
<td>Formalin</td>
<td>1000 µL/dL</td>
</tr>
<tr>
<td>Thymol</td>
<td>50 mg/dL</td>
</tr>
<tr>
<td>Toluene</td>
<td>1000 µL/dL</td>
</tr>
<tr>
<td>Xylene</td>
<td>1000 µL/dL</td>
</tr>
</tbody>
</table>
Precision
Precision

Assay precision is ≤ 5% total CV.

<table>
<thead>
<tr>
<th>AEROSET®</th>
<th>Level</th>
<th>N</th>
<th>Mean µg/mL</th>
<th>Within run SD</th>
<th>Within Run %CV</th>
<th>Total SD</th>
<th>Total %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>84</td>
<td>29.14</td>
<td>0.3405</td>
<td>1.13</td>
<td>0.4981</td>
<td>1.66</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>84</td>
<td>88.58</td>
<td>1.0361</td>
<td>1.21</td>
<td>1.6771</td>
<td>1.96</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>c8000®</th>
<th>Level</th>
<th>N</th>
<th>Mean µg/mL</th>
<th>Within run SD</th>
<th>Within Run %CV</th>
<th>Total SD</th>
<th>Total %CV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>84</td>
<td>30.15</td>
<td>0.2219</td>
<td>0.74</td>
<td>1.1429</td>
<td>3.79</td>
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<tr>
<td></td>
<td>2</td>
<td>84</td>
<td>90.93</td>
<td>0.6764</td>
<td>0.74</td>
<td>1.8850</td>
<td>2.07</td>
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</tbody>
</table>
Method Comparison
# Method Comparison

<table>
<thead>
<tr>
<th></th>
<th>AEROSET® vs Hitachi</th>
<th>c8000® vs AEROSET</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>53</td>
<td>53</td>
</tr>
<tr>
<td>Y-Intercept</td>
<td>2.89 (0.384 to 5.389)</td>
<td>1.3 (-0.297 to 2.897)</td>
</tr>
<tr>
<td>Correlation Coefficient</td>
<td>0.9984</td>
<td>0.9994</td>
</tr>
<tr>
<td>Slope</td>
<td>1.023 (1.007 to 1.039)</td>
<td>1.019 (1.009 to 1.029)</td>
</tr>
</tbody>
</table>
Assay Specific Information
MULTIGENT® Microalbumin

**Assay Method:** immunoturbidimetric

**Reagent list #:** 2K98-20

**Calibration:**
- Spline
- Microalbumin calibrator: 2K98-02

**Assay Stability:**
- Onboard Stability: 28 days (672 hours)
- Calibration Stability: 28 days (672 hours)

**Assay Volumes:**
- Specimen: 6 µL
- R1 Reagent: 180 µL
- R2 Reagent: 30 µL

**Precision:** ≤ 5% Total CV

**Reportable Range:**
5 to 500 µg/mL (mg/L)

**Analytical Sensitivity/Limit of Detection (LOD):**
1.0 µg/mL (mg/L)

**Acceptable Specimen:** urine
- 24 hour or spot

**Acceptable preservatives:**
- 6N HCL
- acetic acid
- chloroform
- formalin
- toluene
- xylene
Troubleshooting Tips
Troubleshooting Tips

- Imprecision
  - Any assay using 340 nm as the primary wavelength is more sensitive to lamp age.
  - View the reaction graph, primary wavelength only
  - Lamp degradation: Progress curve not smooth (exhibits an erratic saw-tooth appearance)
Troubleshooting Tips

Erratic results

- Correct SmartWash™ configuration is critical for the uALB assay (especially the Sample Probe SmartWash) due to the protein contained in the sample
- Verify all SmartWash parameters are configured according to the package insert
- Ensure there is adequate volume of wash solutions on the sample carousel and in reagent supply centers
Questions and Answers
Questions and Answers

Can I use another vendor’s control, or is use of MULTIGENT® Microalbumin controls required?

Bovine or other sourced control material, e.g., Bio-Rad controls, do not work with the MULTIGENT Microalbumin assay.

The control must be human-sourced.

Use MULTIGENT Microalbumin controls, LN 2K98-10.
Questions and Answers

What results impact would I see if frozen reagent is used?

Frozen reagent should not be used. The results would be expected to be decreased due to the deterioration of the protein antibody in the R2 reagent.
If controls are out of range, what troubleshooting should be performed?

1. Check the dryer tip. If the dryer tip is not perfectly aligned, residual water or excess protein can remain in the cuvettes. Align or replace the tip as necessary.

2. Inspect the sample probe for protein build-up. Clean stylette with nozzle cleaning wire or replace as necessary.
Questions and Answers

Is the c8000® data for Between Day precision accurate as listed in the Microalbumin package insert?

The values of 0.000 are correct for Between Day precision on c8000.
Questions and Answers

If method comparison studies are done using spot urine samples, should the Microalbumin result be adjusted using the urine creatinine value?

It is not necessary to apply the correction to the microalbumin comparison. The Microalbumin result can be directly compared to the result from the previous method, provided a similar comparison is made of urine creatinine results to those from the previous method.
Questions and Answers

What material is available to verify the reportable range for Microalbumin?

Customers can dilute a control with saline to create linearity samples.

Quantimetrix Microbumin Microalbumin Control No. 1250-01, 2 Level Set, 10 x 7 mL
www.4qc.com
Questions and Answers

Is it possible to see falsely increased or decreased results with Microalbumin due to non-specific turbidity?

Yes. In some instances. Prozone may be observed at albumin concentrations greater than 10,000 µg/mL. If a result is questionable, dilute the sample and repeat the analysis.
Questions and Answers

Can a diagnosis of microalbuminuria be made using one urine sample?

No. Due to variability in urinary albumin excretion, at least two of three test results measured within a 6-month period should show elevated levels before a patient is designated as having microalbuminuria.
Questions and Answers

Can I use the System to perform the calculation required to report a Microalbumin result on a spot urine?

Yes. Create a panel consisting of Microalbumin, Urine Creatinine, and a calculated assay using the appropriate formula for SI or conventional units. When a spot collection is received, order the panel and report the calculated result as the Microalbumin result.