This package insert must be read carefully prior to product use. Package insert instructions must be followed accordingly. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Key to symbols used

REF List Number

LOT Lot Number

IVD In Vitro Diagnostic Medical Device

Expiration Date

Cuvettes Cuvettes

Consult instructions for use

Dilution Buffer Dilution Buffer

Calibrator (A-F)

Store at 2-8°C

Control Low, Medium, High (L, M, H)

CAUTION: Consult accompanying documents

Reagent Pack

Abbot Laboratories Diagnostics Division

Manufacturer

Authorized Representative

See REAGENTS section for a full explanation of symbols used in reagent component naming.
INTENDED USE

The TDx/TDxFlx Amikacin assay is a reagent system for the quantitative measurement of amikacin, an aminoglycoside antibiotic drug, in serum or plasma. The measurements obtained are used in the diagnosis and treatment of amikacin overdose and in monitoring levels of amikacin to ensure appropriate therapy.

SUMMARY AND EXPLANATION OF TEST

The Amikacin assay utilizes Fluorescence Polarization Immunoassay (FPIA) technology. Refer to your TDx or TDxFlx System Operation Manual in the System Description section for a discussion of this technology.

Amikacin is effective in the treatment of serious gram-negative infections and is particularly useful in those involving strains resistant to all the other aminoglycosides. Amikacin is probably the aminoglycoside of first choice when gentamicin resistance is strongly suspected. As with other aminoglycosides, there is a narrow margin between therapeutic effectiveness and toxicity. Amikacin should be used with caution in patients with depressed renal function. Serum levels should be monitored, applying the concepts developed for gentamicin.

PRINCIPLES OF THE PROCEDURE

Refer to your operation manual in the System Description section.

### REAGENTS

<table>
<thead>
<tr>
<th>Item Contents</th>
<th>Contents</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤10% Amikacin Antiserum (Sheep) in buffer with protein stabilizer (4.0 mL).</td>
<td>S</td>
</tr>
<tr>
<td>&lt;0.01% Amikacin Fluorescein Tracer in buffer containing surfactant and protein stabilizer (3.5 mL).</td>
<td>T</td>
</tr>
<tr>
<td>Pretreatment Solution. Surfactant in buffer containing protein stabilizer (2.5 mL).</td>
<td>P</td>
</tr>
<tr>
<td>≤10% Amikacin Antiserum (Sheep) in buffer with protein stabilizer (4.0 mL).</td>
<td>S</td>
</tr>
<tr>
<td>&lt;0.01% Amikacin Fluorescein Tracer in buffer containing protein stabilizer (2.5 mL).</td>
<td>T</td>
</tr>
<tr>
<td>Pretreatment Solution. Surfactant in buffer containing protein stabilizer (2.5 mL).</td>
<td>P</td>
</tr>
</tbody>
</table>

Amikacin Concentration

<table>
<thead>
<tr>
<th>(μg/mL)</th>
<th>(μmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0</td>
<td>0.00</td>
</tr>
<tr>
<td>3.0</td>
<td>5.12</td>
</tr>
<tr>
<td>10.0</td>
<td>17.08</td>
</tr>
<tr>
<td>20.0</td>
<td>34.15</td>
</tr>
<tr>
<td>35.0</td>
<td>59.77</td>
</tr>
<tr>
<td>50.0</td>
<td>85.38</td>
</tr>
</tbody>
</table>

Preservative: Sodium Azide.

Abbott manufactures internal reference standards for Amikacin using Amikacin Reference Standard (USP Grade). Amikacin calibrators are manufactured gravimetrically and tested against these internal reference standards.

### Controls

<table>
<thead>
<tr>
<th>Target Conc. (μg/mL)</th>
<th>Range (μg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.0</td>
<td>13.50 - 16.50</td>
</tr>
<tr>
<td>30.0</td>
<td>27.00 - 33.00</td>
</tr>
</tbody>
</table>

Preservative: Sodium Azide.
Warnings or Precautions For Users
For In Vitro Diagnostic Use.

**CAUTION:** This product contains human sourced and/or potentially infectious components. Refer to the REAGENTS section of this package insert. No known test method can offer complete assurance that products derived from human sources or inactivated microorganisms will not transmit infection. Therefore, all human sourced materials should be considered potentially infectious. It is recommended that these reagents and human specimens be handled in accordance with the OSHA Standard on Bloodborne Pathogens5. Biosafety Level 2 or other appropriate biosafety practices6, 7 should be used for materials that contain or are suspected of containing infectious agents.

The human serum used in the Calibrators and Controls is nonreactive for HBsAg, HIV-1 RNA or HIV-1 Ag, anti-HCV, and anti-HIV-1/HIV-2.

**TDx/FLx:** Vials in the wedge pack may contain an apparatus that reduces evaporation; DO NOT remove the apparatus.

**CAUTION:** Inversion of the TDx/FLx (wedge) reagent pack may cause liquid entrapment in the snap cap.

**TDx/FLx:** Before each assay run, mix the TDx/FLx (batch) reagent pack by gentle inversion.

This product contains sodium azide; for a specific listing, refer to the REAGENTS section. Contact with acids liberates very toxic gas. This material and its container must be disposed of in a safe way.

For product not classified as dangerous per European Directive 1999/45/EC as amended - Safety data sheet available for professional user on request.

Refer to your operation manual in the System Description section.

**Storage Instructions**

Store reagents, calibrators, and controls refrigerated at 2-8°C (35-46°F).

**Indications of Stability**

Reagents are stable until the expiration date when stored and handled as directed.

**NOTE:** The stability of the reagent is valid for 180 days after the application of the snap cap. Do not use reagents beyond the expiration date printed on the kit label.

For additional information, refer to the Calibration and Quality Control sections in this insert.

---

**INSTRUMENT PROCEDURE**

**Assay Parameters Amikacin**

The Amikacin assay parameters are factory set for twenty-one (21) different functions. Refer to your operation manual in the Operation section for an explanation of assay parameters.

Before beginning the initial calibration and subsequent analysis with the Amikacin assay, press ASSAY, 3 and PRINT on the control panel. Verify the parameters with the following assay illustration.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPL VOL</td>
<td>1.2</td>
</tr>
<tr>
<td>SPL REP</td>
<td>1</td>
</tr>
<tr>
<td>LULUM</td>
<td>0.00</td>
</tr>
<tr>
<td>HILUM</td>
<td>50.00</td>
</tr>
<tr>
<td>CAL VOL</td>
<td>1.2</td>
</tr>
<tr>
<td>CAL REP</td>
<td>2</td>
</tr>
<tr>
<td>CONC A</td>
<td>0.00</td>
</tr>
<tr>
<td>CONC B</td>
<td>3.00</td>
</tr>
<tr>
<td>CONC C</td>
<td>10.00</td>
</tr>
<tr>
<td>CONC D</td>
<td>20.00</td>
</tr>
<tr>
<td>CONC E</td>
<td>35.00</td>
</tr>
<tr>
<td>CONC F</td>
<td>50.00</td>
</tr>
<tr>
<td>UNITS</td>
<td>0</td>
</tr>
<tr>
<td>CRV FIT</td>
<td>2</td>
</tr>
<tr>
<td>MIX DEV</td>
<td>5.0</td>
</tr>
<tr>
<td>MN FOLIA</td>
<td>*</td>
</tr>
<tr>
<td>MN SPAN</td>
<td>#</td>
</tr>
<tr>
<td>MODE</td>
<td>1</td>
</tr>
<tr>
<td>GAIN</td>
<td>*</td>
</tr>
<tr>
<td>MIX BKG</td>
<td>1800.00</td>
</tr>
<tr>
<td>MN TR</td>
<td>*</td>
</tr>
</tbody>
</table>

To edit a parameter, follow these steps:

1. Press ASSAY, 3, . . . plus the parameter number you wish to change, and EDIT.
2. Enter the new parameter value. Press STORE.
3. Press NEXT to continue to the next parameter. Press STORE after each parameter is edited.
4. After editing has been completed, press STOP.

The instrument will return to operational status and the display panel will read: READY

**NOTES:**

* Parameters cannot be edited.
# Parameter can be edited and may vary with reagent pool changes.

Refer to your operation manual for a discussion of:

- Quality Control
- Installation Procedures
- Methods of Operation
- Performance Characteristics
- Assay Procedures
- Barcode Override
- Dilution Protocol
- Calibration Procedures
- Operational Precautions and Limitations
- Maintenance and Component Replacement
SAMPLE COLLECTION AND PREPARATION FOR TESTING ANALYSIS

Samples should be reconstituted upon collection and stored frozen if not analyzed within 24 hours. Complete mixing of each thawed sample is required before analysis.

Samples containing additional antibiotics should be stored frozen if a delay in analysis of more than 8 hours is anticipated. The samples should be frozen -8°C to -70°C. Failure to freeze serum samples containing additional antibiotics may result in falsely low amikacin levels due to in vitro inactivation.

Serum or plasma specimens may be used with the Amikacin assay. For additional information, refer to your operation manual in the System Description section.

Interferences

The compounds listed below, added to human serum, resulted in less than 5% error in detecting added drug.

- Bupivacaine
- Cholesterol
- Hemoglobin
- Heparin
- Total Protein
- Triglycerides

To determine whether recalibration is required, this reagent system should be checked by assaying all the controls. If the control results are within range, patient samples may be run without need for recalibration. If the control results are not within range, refer to your operation manual in the Troubleshooting section.

Quality Control

The recommended control requirement is one Amikacin control level tested once every 8 hour shift, no less than two different controls per 24 hours which span the medical decision range. Controls should be run on all patient samples and may be placed in any position on the Sample Carousel. If the quality control procedures in your laboratory require more control testing to verify test results, follow those procedures.

Ensure that assay control values are within the concentration ranges specified in the REAGENTS section of this insert.

At the discretion of the laboratory, selected quality control rules may be applied to the quality control data. As an example, a Levey-Jennings plot may be used to track the assay’s performance. Refer to your laboratory Standard Operating Procedure and/or Quality Assurance Plan for additional details.

When an assay control does not meet the established criteria for acceptability, associated test results should be considered suspect. Your laboratory should evaluate results and take remedial corrective action prior to reporting test results. In conjunction with your laboratory’s Quality Assurance Plan, refer to your operation manual in the Troubleshooting section.

RESULTS

The TDx and TDxFLx software calculates a best-fit curve equation that is used to generate a calibration curve. This curve is stored in memory and concentrations of drug in unknown samples are calculated from this curve using polarization values generated for each sample in the assay.

For additional details in reporting results refer to your operation manual in the Operation section for printouts.

LIMITATIONS OF THE PROCEDURE

As with all analyte determinations, the Amikacin value should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

Patient samples which contain kanamycin A, kanamycin B, 9’, or 4’ dideoxykanamycin B will yield falsely elevated values for amikacin. However, these drugs are not usually costimulated with amikacin.

High concentration of penicillin or cephalosporin have been shown to inactivate amnoglycosides in vitro. The degree of inactivation is dependent on the particular amnoglycoside being measured, the penicillin or cephalosporin concentration and the storage conditions of the sample.

Samples from patients receiving antibiotics should be frozen immediately or stored frozen.

The concentration range of the XSYSTEMS Amikacin Calibrators (0.0 - 10.0 μg/mL) is adequate to accurately determine the Amikacin concentration in most of the patient samples encountered. Occasionally, samples contain amikacin in concentrations greater than the highest calibrator (10.0 μg/mL). To accurately determine the drug concentration in these samples, the Dilution Protocol described in the Operation Section of the operation manual may be used with both the batch and random access option.

A manual dilution may also be used to determine the drug concentration in elevated samples. Dilute the sample with XSYSTEMS Dilution Buffer and repeat the test using the manual dilution sample. The concentration reported by the instrument must be multiplied by the manual dilution factor to obtain the final sample concentration.

The concentration range of the XSYSTEMS Amikacin Calibrators (0.0 - 10.0 μg/mL) is adequate to accurately determine the Amikacin concentration in most of the patient samples encountered. Occasionally, samples contain amikacin in concentrations greater than the highest calibrator (10.0 μg/mL). To accurately determine the drug concentration in these samples, the Dilution Protocol described in the Operation Section of the operation manual may be used with both the batch and random access option.

A manual dilution may also be used to determine the drug concentration in elevated samples. Dilute the sample with XSYSTEMS Dilution Buffer and repeat the test using the manual dilution sample. The concentration reported by the instrument must be multiplied by the manual dilution factor to obtain the final sample concentration.
EXPECTED VALUES
Strong correlations have been shown between serum levels and both therapeutic effect and toxicity in specific patient types. Peak serum levels of amikacin in the range of 20 to 25 μg/mL are suggested for optimal therapeutic effectiveness. Persistently elevated peak concentrations (30-35 μg/mL) have been shown to cause renal and central nervous system toxicity. Nephrotoxicity takes the form of damage to the proximal renal tubules, and is associated with impaired renal function. Central nervous system toxicity is most often manifested as damage to the vestibular and auditory branches of the eighth cranial nerve. Trough levels offer a more discrete indication of impending toxicity since they more closely correspond to tissue levels and are less affected by sampling errors. Slowly rising trough levels have been shown to correspond to tissue accumulation of the drug, and trough levels greater than 10 μg/mL have been associated with renal failure in some patients.

SPECIFIC PERFORMANCE CHARACTERISTICS
Specificity
Cross-reactivity was tested for compounds whose chemical structure or concurrent usage could cause concern over potential interference with the Amikacin assay.

The aminoglycosides, kanamycin, cross-react with the Amikacin assay due to its structural similarity to amikacin. Therefore, the results of this assay cannot be used to accurately quantify amikacin serum or plasma levels in patients using both amikacin and kanamycin. Other antibiotics, didekacin, tobramycin, neomycin, gentamicin, and vancomycin were tested with the Amikacin assay at the upper limit of their respective therapeutic ranges; none of these compounds registered values above the sensitivity of the Amikacin assay.

The following compounds showed less than 1% cross-reactivity when tested with the Amikacin assay.

Cross-reactivity levels were tested using drug-free samples for compounds listed. Percent cross-reactivity = 100 x ("concentration found" divided by "concentration added"). Cross-reactivity was tested for compounds whose chemical structure or concurrent usage could cause concern over potential interference with the Amikacin assay.

Specificity
Cross-reactivity was tested for compounds whose chemical structure or concurrent usage could cause concern over potential interference with the Amikacin assay.

The aminoglycosides, kanamycin, cross-react with the Amikacin assay due to its structural similarity to amikacin. Therefore, the results of this assay cannot be used to accurately quantify amikacin serum or plasma levels in patients using both amikacin and kanamycin. Other antibiotics, didekacin, tobramycin, neomycin, gentamicin, and vancomycin were tested with the Amikacin assay at the upper limit of their respective therapeutic ranges; none of these compounds registered values above the sensitivity of the Amikacin assay.

The following compounds showed less than 1% cross-reactivity when tested with the Amikacin assay.

Amphotericin Lincomycin
Ampicillin Methicillin
Carbenicillin Methotrexate
Cefadroxil Methylprednisolone
Ciprofloxacin Nalidixic Acid
Cephalaxin Chloramphenicol
Cephaloglycin Penicillin V
Cephaloridine Phenindione
Cephaloridine Phenytoin
Cephalotaxine Pilocarpine
Cephalothin Sarcosine Sulfate
Chloramphenicol Sulfamethoxazole
Clindamycin Sulfasalazine
Erythromycin Specinomycin
Ethacrynic Acid Streptomycin
5-Fluorouracil Sulfadiazine
Forticin A Sulfamethoxazole
Forticin B Tetracycline
Fusidic Acid Ticarcillin Disodium
Fusidic Acid Trimethoprim

Sensitivity
Sensitivity is defined as the lowest measurable concentration which can be distinguished from zero with 95% confidence and was determined to be 0.8 μg/mL.

Precision
Precision was determined as described in National Committee for Clinical Laboratory Standards (NCCLS) protocol EP5-T13 using human serum with 5.0, 15.0, and 30.0 μg/mL of amikacin added. Results from three studies typically yielded CV’s of less than 5%. Representative data follow.

<table>
<thead>
<tr>
<th>Target value (μg/mL)</th>
<th>Expected (Added) (μg/mL)</th>
<th>Recovered (μg/mL)</th>
<th>Percent (%) Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.0</td>
<td>5.0</td>
<td>3.08</td>
<td>102.7</td>
</tr>
<tr>
<td>10.0</td>
<td>10.0</td>
<td>10.06</td>
<td>100.6</td>
</tr>
<tr>
<td>15.0</td>
<td>15.0</td>
<td>15.63</td>
<td>96.9</td>
</tr>
<tr>
<td>20.0</td>
<td>20.0</td>
<td>19.42</td>
<td>97.1</td>
</tr>
<tr>
<td>30.0</td>
<td>30.0</td>
<td>30.70</td>
<td>102.3</td>
</tr>
<tr>
<td>35.0</td>
<td>35.0</td>
<td>34.42</td>
<td>98.3</td>
</tr>
</tbody>
</table>

Accuracy by Recovery
Recovery was determined by adding amikacin to human serum at clinically relevant concentrations and assaying in replicates of five. Recoveries were found to be quantitative.

Accuracy by Correlation with Reference Assays
The Amikacin assay was compared to commercially available kits by assaying clinical samples obtained from patients on Amikacin therapy. Representative results follow.

<table>
<thead>
<tr>
<th>TDX vs. TDx FLx Batch</th>
<th>Number of Observations</th>
<th>Intercept</th>
<th>Slope</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIA A</td>
<td>143</td>
<td>0.38</td>
<td>0.914</td>
<td>0.956</td>
</tr>
<tr>
<td>RIA B</td>
<td>162</td>
<td>-0.264</td>
<td>0.887</td>
<td>0.971</td>
</tr>
<tr>
<td>Fluorescence Immunoassay</td>
<td>92</td>
<td>0.003</td>
<td>0.990</td>
<td>0.977</td>
</tr>
</tbody>
</table>

The Amikacin assay was performed in three modes of operation: TDX, TDx FLx Batch, and TDx FLx Random Access. The following data are representative comparisons between these modes.

<table>
<thead>
<tr>
<th>TDX FLx Random Access vs. TDX Batch</th>
<th>Number of Observations</th>
<th>Intercept</th>
<th>Slope</th>
<th>Correlation Coefficient</th>
</tr>
</thead>
<tbody>
<tr>
<td>99</td>
<td>-0.14</td>
<td>1.03</td>
<td>0.997</td>
<td></td>
</tr>
<tr>
<td>99</td>
<td>-0.15</td>
<td>1.07</td>
<td>0.998</td>
<td></td>
</tr>
<tr>
<td>99</td>
<td>0.04</td>
<td>1.02</td>
<td>0.998</td>
<td></td>
</tr>
</tbody>
</table>
REFERENCES

11. Arlett JP. Interpretation of antimicrobial concentrations in serum. American Association for Clinical Chemistry Therapeutic Drug Monitoring Continuing Education and Quality Control Program 1981; April 1-11.

TDXs and TDXFLx are trademarks of Abbott Laboratories.