

Thermo Scientific QMS[®] Amikacin and Gentamicin Immunoassays on Automated Analyzers

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Abstract

Background: Amikacin and gentamicin are aminoglycoside antibiotics. Amikacin, a semi-synthetic antibiotic, is effective against a wide range of pathogens. Trough values in the range of 5.0 to 10.0 µg/mL are accepted for therapeutic effectiveness for amikacin. Gentamicin, isolated from bacteria of the genus *Micromonospora*, is effective against many gram-negative bacteria as well as certain gram-positive species. Therapeutic range for gentamicin is 2.0 to 8.0 µg/mL. Kidney damage and hearing loss are the most important side-effects for both amikacin and gentamicin. Because of this potential, blood levels of the drug may be monitored.

Objective: The aim of the study was to evaluate the performance of the Thermo Scientific QMS Amikacin Immunoassay and the Thermo Scientific QMS Gentamicin Immunoassay and compare the results to those generated by the previously approved FPIA method.

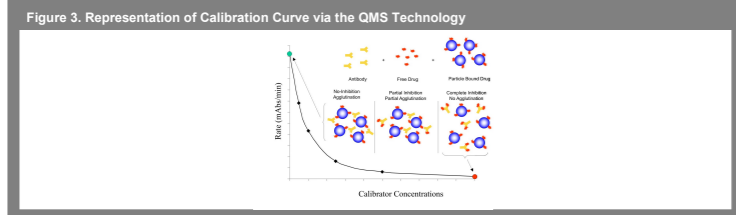
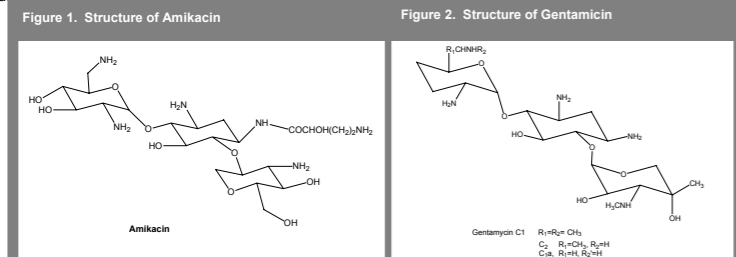
Methods: The QMS Amikacin and Gentamicin Immunoassays are homogeneous particle-based turbidimetric agglutination immunoassays. These assays are competitive (free analyte such as amikacin or gentamicin, competitor or conjugate on the particle and antibody). A standard curve can be generated with the highest rate of agglutination at the lowest analyte concentration and the lowest agglutination rate at the highest analyte concentration. The QMS Amikacin and Gentamicin Immunoassays are calibrated with six-point calibration (0.0, 3.0, 10.0, 20.0, 35.0 and 50.0 µg/mL for amikacin; 0.0, 0.5, 1.5, 3.0, 6.0, 10.0 µg/mL for gentamicin). The LDD of the QMS Amikacin Immunoassay was determined to be 0.8 µg/mL. The LOQ of the QMS Gentamicin Immunoassay was determined to be 0.3 µg/mL. The assay range is 1.5 to 50.0 µg/mL for the QMS Amikacin Immunoassay and 0.3 to 10.0 µg/mL for the QMS Gentamicin Immunoassay. Precision was determined as described in NCCLS protocol EP5-A. For the QMS Amikacin Immunoassay, Within Run precision ranged from 1.79% to 5.37%. Between Day precision ranged from 0.70% to 4.77%. The Total CV ranged from 6.22% to 9.94%. For the QMS Gentamicin Immunoassay, Within Run precision ranged from 1.30% to 3.70% and Between Day precision ranged from 1.40% to 2.80%. The Total CV ranged from 2.80% to 3.90%. The QMS Amikacin and Gentamicin Immunoassays were compared to the equivalent FPIA using the Passing-Bablok regression analysis. Testing of 56 samples in the comparison amikacin study showed a slope of 1.00, y-intercept of 0.25 and correlation coefficient (R²) of 0.992. Results of 63 samples in the gentamicin comparison study gave a slope of 1.102, y-intercept of -0.412 and correlation coefficient (R²) of 0.997.

Conclusion: The data indicated that the QMS Immunoassays are sensitive, accurate, reproducible and can provide useful guidance in therapeutic management of aminoglycoside antibiotics.

Introduction

Amikacin (Figure 1) is a semi-synthetic aminoglycoside that exhibits bactericidal activity against a wide range of pathogens, including many organisms resistant to other aminoglycosides.¹⁻⁴ Gentamicin (Figure 2), isolated from bacteria of the genus *Micromonospora*, is effective against many gram-negative bacteria as well as certain gram-positive species. Gentamicin has a narrow range of safe and effective concentrations. Exposure to high concentrations for a prolonged period may cause renal impairment or ototoxicity.⁵ Kidney damage and hearing loss are the most important side-effects for both amikacin and gentamicin. Because of this potential, blood levels of the drug should be monitored.

QMS Immunoassays are homogeneous immunoassays for the quantitative determination of the aminoglycoside antibiotics (amikacin and gentamicin) in human serum or plasma. Monitoring the concentrations of aminoglycoside antibiotics (amikacin and gentamicin) in human serum or plasma, along with careful clinical assessment, is the most effective means of ensuring adequate therapy.



Methods & Materials

QMS Immunoassays are homogeneous particle-based turbidimetric agglutination immunoassays. These assays are competitive (free analyte such as amikacin or gentamicin, competitor or conjugate on the particle and antibody). A standard curve can be generated with the highest rate of agglutination at the lowest analyte concentration and the lowest agglutination rate at the highest analyte concentration (Figure 3). The QMS Amikacin and Gentamicin Immunoassays are calibrated with six-point calibration (0.0, 3.0, 10.0, 20.0, 35.0 and 50.0 µg/mL for amikacin; 0.0, 0.5, 1.5, 3.0, 6.0 and 10.0 µg/mL for gentamicin).

Results

Specificity:

Interfering Substances

The following compounds shown in Table 1 and Table 2, when tested with the QMS Amikacin and Gentamicin Immunoassays at the concentrations indicated, resulted in less than 10% error in detecting amikacin or gentamicin. Interference studies were conducted using NCCLS protocol EP7-A.⁶

Drug Cross-Reactivity

Cross-reactivity was tested with drugs that are routinely administered with amikacin or gentamicin. The following compounds in Table 3 and in Table 4 were tested.

Table 1. Interfering Substances (Amikacin)

Interfering Substance	Interferent Concentration	N	Amikacin (µg/mL)	Recovery
Total Protein	12 g/dL	3	24.03	96.00%
Bilirubin	15 mg/dL	2	21.65	96.40%
Hemoglobin	10 g/L	2	17.32	93.42%
HAMA Type-1	Normal human level	2	20.41	100.49%
HAMA Type-2	Normal human level	2	16.98	98.04%
Triglyceride	1691 mg/dL	3	24.03	96.30%

Table 2. Interfering Substances (Gentamicin)

Interfering Substance	Interferent Concentration	N	Gentamicin (µg/mL)	Recovery
Bilirubin	20 mg/dL	3	3.44	100.00%
HAMA type-1	Normal human level	2	3.33	100.00%
HAMA type-2	Normal human level	2	3.33	94.00%
Hemoglobin	2 g/dL	3	3.44	99.00%
Rheumatoid Factor	1,240 IU	3	3.56	98.00%
Total protein	12 g/dL	3	3.44	93.00%
Triglyceride	1691 mg/dL	3	3.44	96.00%

Table 3. Specificity (Amikacin)

Compound	Compound Concentration (µg/mL)	Amikacin Concentration (µg/mL)	Cross-Reactivity*
5-Fluorocytosine	30	19.82	-0.39%
Amphotericin	100	20.92	1.33%
Ampicillin	50	19.50	ND
Carbencillin	2500	20.25	ND
Cephalexin	320	20.16	ND
Cephalosporin C	1000	19.16	ND
Cephalothin	1000	20.79	ND
Chloramphenicol	250	20.98	0.55%
Clindamycin	2000	18.44	ND
Erythromycin	500	19.71	ND
Ethacrynic acid	400	20.77	ND
Furosemide	100	20.60	1.00%

ND= Non-detectable

Table 4. Specificity (Gentamicin)

Compound	Compound Concentration (µg/mL)	Gentamicin Concentration (µg/mL)	Cross-Reactivity*
Acetaminophen	200	1.58	ND
Acetyl cysteine	1000	3.71	ND
Acetylsalicylic acid	300	1.56	ND
Amikacin	300	3.76	ND
Amphotericin B	100	3.71	ND
Ampicillin	50	3.76	ND
Ascorbic Acid	30	3.76	-0.23%
Carbencillin	2500	3.71	ND
Cefamandole	250	3.76	ND
Nafate			

ND= Non-detectable

Precision:

Precision was determined as described in NCCLS protocol EP5-A.⁷ Tri-level human serum based commercial controls containing amikacin and gentamicin were used in the study. Each level of the control was assayed in duplicate twice a day for 20 days. Each of the runs per day was separated by at least two hours. The means along with the Within Run, Between Day, Total SD and percent CVs were calculated. Representative results are shown in Table 5 and Table 6.

Table 5. Precision summary (Amikacin)

Amikacin Sample	N	Within Run			Between Day		Total	
		Mean (µg/mL)	SD	CV	SD	CV	SD	CV
1	80	4.09	0.22	5.37%	0.19	4.77%	0.41	9.94%
2	80	12.00	0.21	1.79%	0.08	0.70%	0.74	6.22%
3	80	24.37	0.47	1.93%	0.40	1.65%	1.54	6.32%

Acceptance criteria: <10% total CV

Table 6. Precision summary (Gentamicin)

Gentamicin Sample	N	Within Run			Between Day		Total	
		Mean (µg/mL)	SD	CV	SD	CV	SD	CV
1	80	2.45	0.09	3.70%	0.03	1.40%	0.10	3.90%
2	80	6.10	0.12	2.00%	0.17	2.80%	0.22	3.60%
3	80	9.12	0.12	1.30%	0.19	2.00%	0.25	2.80%

Acceptance criteria: <10% total CV

Assay Range:

The range of the Amikacin QMS Immunoassay is 1.5 to 50.0 µg/mL. The range of the Gentamicin QMS Immunoassay is 0.3 to 10.0 µg/mL.

Linearity:

Each level of the QMS Amikacin calibrators and the QMS Gentamicin calibrators was serially diluted and run in triplicate. A mean of the replicates for each sample was determined and a percent recovery was calculated. Results are shown in Table 7 and Table 8.

Table 7. Linearity (Amikacin)

Theoretical Concentration (µg/mL)	Mean Recovered Concentration (µg/mL)	Recovery
1.5	1.67	111.30%
6.5	6.48	99.70%
15	14.67	97.80%
27.5	26.32	95.70%
42.5	41.44	97.50%

Mean recovery: 100.40%

Table 8. Linearity (Gentamicin)

Theoretical Concentration (µg/mL)	Mean Recovered Concentration (µg/mL)	Recovery
1.72	1.64	95.00%
3.44	3.62	105.00%
5.16	5.27	102.00%
6.88	7.20	105.00%

Mean recovery: 102.00%

Sensitivity:

Least Detectable Dose (LDD) of the QMS Amikacin Immunoassay is defined as the lowest measurable concentration that can be distinguished from zero with 95% confidence. The LDD was determined to be 0.8 µg/mL. **Limit of Quantitation (LOQ)** of the QMS Gentamicin Immunoassay is defined as the lowest concentration of an analyte that can be reliably detected and at which the total error meets accuracy requirements. The LOQ was determined to be 0.3 µg/mL.

Method Comparison:

Correlation studies were performed using NCCLS Protocol EP9-A.⁸ Results from the QMS Amikacin Immunoassay and the QMS Gentamicin Immunoassay were compared with results from a commercially available FPIA. The patient samples consisted of serum and plasma. The amikacin. Results of the Passing-Bablok⁹ regression analysis for the study are shown in Table 9 and Table 10.

Table 9. Method Comparison (Amikacin)

Amikacin Assays	
Slope	1
y-intercept	0.25
Correlation Coefficient	0.992
Number of Samples	56

Table 10. Method Comparison (Gentamicin)

Gentamicin Assays	
Slope	1.102
y-intercept	-0.412
Correlation Coefficient	0.997
Number of Samples	63

Conclusion

The data indicates that the QMS Amikacin Immunoassay and the QMS Gentamicin Immunoassay are sensitive, accurate and reproducible assays that can provide useful guidance in therapeutic management of aminoglycoside antibiotics.

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