

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k043384

B. Purpose for Submission:

New assay and calibrators. Changes to calibrator verifiers.

C. Measurand:

Tobramycin

D. Type of Test:

Quantitative homogeneous enzyme immunoassay

E. Applicant:

Ortho-Clinical Diagnostics Inc.

F. Proprietary and Established Names:

VITROS Chemistry Products Tobramycin Reagent, Calibrator Kit 14, TDM Performance Verifier I, II and III.

G. Regulatory Information:

1. Regulation section:
Tobramycin Test System (21CFR862.3900)
Assayed controls (21 CFR 862.3280)
Calibrators (21 CFR 862.3200)
2. Classification:

Class II
3. Product code:

91LDO, 91DLJ, 91DIF

4. Panel:

91, Toxicology

H. Intended Use:

1. Intended use(s): See indications for use below.

2. Indication(s) for use:

For *in vitro* diagnostic use only. VITROS Chemistry Products TOBRA Reagent is used on the VITROS FS 5,1 Chemistry System to quantitatively measure tobramycin (TOBRA) concentration in human serum and plasma. Serum or plasma tobramycin measurements are used in the diagnosis and treatment of tobramycin overdose and in monitoring levels of tobramycin to help ensure appropriate therapy.

For *in vitro* diagnostic use only. VITROS Chemistry Products Calibrator Kit 14 is used to calibrate VITROS 5,1 FS Chemistry Systems for the quantitative measurement of tobramycin (TOBRA).

For *in vitro* diagnostic use only. VITROS TDM Performance Verifier is an assayed control used to monitor performance of ACET, CRBM, DGXN, PHBR, PHYT, and TOBRA on VITROS Chemistry Systems.

3. Special conditions for use statement(s):

See specificity concerning drugs that may interfere with the assay. The device is for use with serum and plasma (lithium heparin and EDTA). It is not for use with other anticoagulants.

4. Special instrument requirements: For use on the VITROS 5,1 FS

I. Device Description:

Reagent 1 contains tobramycin labeled with glucose-6-phosphate dehydrogenase and other non-reactive components, including mouse monoclonal antibodies. Reagent 2 contains polyclonal sheep antibodies to tobramycin, NAD, glucose-6-phosphate and other non-reactive components.

Calibrator Kit 14 is an aqueous solution containing tobramycin, buffer, proteins, salts, surfactants and preservatives. Nominal values of tobramycin (ug/mL): 0, 0.6, 2, 4, 6, 10.

The performance verifiers are assayed controls prepared from bovine serum to which therapeutic drugs, salts and preservatives are added.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Syva[®] EMIT[®] 2000 Tobramycin Assay and calibrators; VITROS Chemistry Products Performance Verifiers

2. Predicate 510(k) number(s):

k003341 (reagents), k042476 (performance verifiers)

3. Comparison with predicate:

The devices are similar in intended use and methodology. Both devices are homogeneous enzyme immunoassays. The predicate device is for use on Syva Analyzer Systems; the new device is for use on the VITROS 5,1 FS Chemistry System.

The VITROS Chemistry Products TDM Performance Verifiers are substantially equivalent to VITROS Chemistry Products TDM Performance Verifiers, currently in commercial distribution (K042476). Tobramycin has been added and labeling updated to add assigned values.

K. Standard/Guidance Document Referenced (if applicable):

NCCLS Guideline – EP5-A, Evaluation of Precision Performance of Clinical Chemistry Devices

NCCLS Guideline – EP6-A, Evaluation of the Linearity of Quantitative Analytical Methods

NCCLS Guideline – EP7-A, Interference Testing in Clinical Chemistry

NCCLS Guideline – EP9-A2, Method Comparison and Bias Estimation Using Patient Samples

L. Test Principle:

Patient sample is added to reagent 1, which contains tobramycin labeled with glucose-6-phosphate dehydrogenase, followed by reagent 2, which contains antibody reactive to tobramycin, glucose-6-phosphate and NAD. The assay is based on competition between tobramycin in the sample and labeled tobramycin. Tobramycin in the sample, related to enzyme activity, is monitored spectrophotometrically at 340 nm. Unknown sample concentrations are determined using the (stored) calibration curve.

M. Performance Characteristics (if/when applicable):

Analytical studies were performed on 3 lots.

1. Analytical performance:

a. *Precision/Reproducibility:*

Within-day and within laboratory precision was determined using the system's bovine serum-based QC materials. The evaluation followed NCCLS EP-5A, with 2 replicates per run, two runs per day for 22 days, n=88 observations. Within-day runs were separated by at least 2 hours. Calibration was performed once each week. The sample order was randomized. Results for within-day and within-lab are shown. Testing of multiple lots and instruments yielded similar results.

Sample	Mean (ug/mL)	N	Within-day SD (ug/mL)	Within-lab (ug/mL)	Within-lab %CV
Control level 1	1.35	88	0.035	0.053	3.9
Control level 2	3.44	88	0.049	0.082	2.4
Control level 3	7.12	88	0.054	0.112	1.6

Within-run precision was also estimated for patient serum pools at concentrations near the low end of the reportable range. Standard deviations were calculated based on 5 replicates, for each of 3 reagent lots, at tobramycin levels ranging from 0.36-1.1 ug/mL (i.e. total of 15 observations at each level). Results near the limit of detection were all within the within-run standard deviation acceptance limits of 0.088 ug/mL.

b. *Linearity/assay reportable range:*

The evaluation followed NCCLS EP-6A. Serum pools with tobramycin concentrations at 12 levels spanning the reportable range were evaluated. Each level was tested in replicates of 5 and average values of observed/expected concentrations were determined. The assay reportable range is 0.6-10 ug/mL. In this range deviations from expected concentrations were less than the bias limits of +/-0.26 ug/mL near the low end and +/-1.3 ug/mL near the high end of the reportable range.

Recovery after sample dilution with the recommended diluent was also evaluated. Serum samples with tobramycin concentrations in the range of approximately 8 ug/mL were diluted 2x and 4x. Recoveries (from triplicate analyses) were all within acceptance criteria of 88-112%.

- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
VITROS Chemistry Products Calibrator Kit 14 and performance verifiers I, II and III are for use with this device.

Value assignment for calibrators: Values assigned to calibrators are traceable to USP tobramycin reference standard. Stock solution concentrations are confirmed by GCMS or HPLC methods. Expanded uncertainties for product calibrators (the 95% confidence interval around the assigned value mean) are 0.09 for 0.6 ug/mL and 0.41 for 10 ug/mL. Correlation analyses (described in the 510(k)) to confirm that trueness is transferred, indicate slopes and intercepts approaching 1 and 0, respectively, and correlation coefficients > 0.99.

Calibrator long-term stability: Calibrators are stored at 9 degrees C and evaluated at intervals up to and past the expiration date. Testing compares results obtained using the test calibrators to those obtained using reference calibrators, on the Syva 30R Analyzer. The samples used in testing were sample pools at medical decision points, and bias is determined for the reference versus the test calibrators. Acceptance criteria for bias are:
+/- 0.169 ug/mL for tobramycin concentrations < 2 ug/mL, and
+/-0.0754[tobramycin] + 0.0184 ug/mL for concentrations > 2 ug/mL.

Calibrator opened-bottle stability: Opened bottle stability is evaluated at intervals up to and past recommended expiration date and restored at 2-8 degrees C. Bias is determined relative to previously unopened calibrators. Acceptance limits are:
+/- 0.069 ug/mL for tobramycin concentrations < 2 ug/mL, and
+/-0.0307[tobramycin] + 0.0075 ug/mL for concentrations > 2 ug/mL.

Value assignment for calibrator verifiers: Performance verifier lots are tested with each reagent to establish target values. Allowable ranges applied reflect estimates of expected lab to lab variability. The range of means is calculated from precision data from multiple systems (minimum 5) in multiple laboratories. A pooled SD is determined based on total SD's from each site and the range is calculated based on 3x pooled SD. Range of means for controls are provided on performance verifier assay sheets.

Verifier stability: Long-term stability for controls is evaluated using vials stored at -18 degrees C at intervals up to and past the expiration date. Opened stability is evaluated using vials opened and stored at 2-8 degrees C up to 7 days. Observed tobramycin concentrations (mean of replicates) are compared to those at "time 0", at intervals up to and past expiration. Examples of calculated acceptance criteria, in terms of bias, relative to time 0, are shown:

	Long-term acceptance limit (ug/mL) (+/-)	Opened vial acceptance limit (ug/mL) (+/-)
Level 1 2 ug/mL	0.35	0.18
Level 2 4 ug/mL	0.69	0.32
Level 3 7 ug/mL	1.21	0.54

d. Detection limit:

The limit of detection, 0.6 ug/ml, was determined based on tobramycin-negative serum samples from 10 human donors, as well as from low level calibrator material. Three reagent lots, 2 calibrator kit lots and 2 instrument systems, were used in testing. The lower limit of detection was calculated as:

$$3.3X\sqrt{\text{Calibration Error Variance (SD)}^2 + \text{Pooled replicate Variance (SD)}^2}$$

Results based on these calculations support the detection limit of 0.6 ug/ml.

The limit for deviation from linearity at samples near the low end of the reportable range is 0.26 ug/mL (see Linearity, above.) Within-run standard deviations were 0.088 ug/mL for samples with concentrations near the low end of the reportable range (see Precision, above.)

e. Analytical specificity:

Testing followed NCCLS EP-7A for the paired-difference method. Human serum pools with tobramycin concentrations approximately 4 and 9 ug/mL, were individually spiked with bilirubin (45 mg/dL), intralipid (1000 mg/dL) and hemoglobin (1000 mg/dL).

Serum pools containing 4 ug/mL tobramycin were spiked with other commonly co-administered drugs and tested for interference. Concentrations of drugs tested were as follows: carbenicillin, 500 ug/mL; cephalothin, 500 ug/mL; chloramphenicol, 1000 ug/mL; clindamycin, 500 ug/mL; erythromycin, 1000 ug/mL; neomycin, 100 ug/mL; netilmicin, 100 ug/mL; penicillin G, 500 ug/mL; sisomicin, 100 ug/mL; streptomycin, 100ug/mL; sulfamethoxazole, 600 ug/mL; tetracycline, 500 ug/mL; trimethoprim, 25 ug/mL and vanomycin, 200 ug/mL.

Bias was calculated as the mean tobramycin concentration observed for the test substance pool minus the mean tobramycin concentration of the control pool. Acceptance criteria are bias < 1.08 ug/mL for the 9 ug/ml pool and bias < 0.5 ug/mL for the 4 ug/mL pool. Based on the criteria above, the drugs listed above did not interfere under the conditions tested. However, high intralipid concentrations may cause sample turbidity and

an absorbance value that exceeds 3.0 AU, causing an analyzer condition code and suppressed results.

The drugs shown in the table below cross-reacted. Percent cross-reactivity is defined: (mean concentration with substance – mean concentration without substance) X (100) /(concentration of substance). Results for samples from patients receiving these drugs may be falsely elevated.

Drug	% cross-reactivity	Tobramycin concentration (ug/mL)
Amikacin	1%	7
Dibekacin	55%	4
Gentamicin	2%	4
Kanamycin	23%	7

f. Assay cut-off:

NA. This is a quantitative assay.

2. Comparison studies:

a. Method comparison with predicate device:

One hundred and eleven human serum samples were evaluated with the VITROS Chemistry Products Tobramycin Reagent and the Syva EMIT[®] Tobramycin Plus. Samples were selected to be patient serum samples of 2 mL or more that contained tobramycin across the reportable range of the assay. Each sample was measured in triplicate. The analysis was also performed using singlicates and results of this analysis are shown below. Similar results were obtained for 3 lots tested. Slope = 1.02, intercept = 0.0, r= 0.99, sy/x = =0.26.

b. Matrix comparison:

Serum and plasma (EDTA and lithium heparin) samples were evaluated by paired difference testing of samples ranging from approximately 1-9 ug/mL. The bias between the mean values of replicates (n=3) was defined as: bias = test sample-serum sample.

Biases observed ranged from approximately -3% to -2% for lithium heparin plasma tubes and -10% to 2% for full EDTA plasma tubes and were within acceptance limits. Results support use of the assay with serum and plasma (EDTA and lithium heparin) samples. The device is not for use with other anticoagulants.

3. Clinical studies:

a. *Clinical Sensitivity:*

N/A. (Not typically reviewed for this type of test)

b. *Clinical specificity:*

N/A. (Not typically reviewed for this type of test)

c. *Other clinical supportive data (when a. and b. are not applicable):*

4. Clinical cut-off:

N/A. See expected values

5. Expected values/Reference range:

Guidelines for reference ranges from the literature are provided.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.