Syva Creatinine Validity Test: Periodic Reverification on The Hitachi 717 Chemistry Analyzer

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Contents

1 Purpose

An annual reverification of the Syva Creatinine Validity Test is performed to establish that the analytical methodology remains valid.

2 Instrumentation and Parameters

A Hitachi 717 running System FD version 7176000-04-07 with Data FD version 7176001-00-01 was used to analyze study samples. Data processing and calculations were performed with the R software environment version 2.8.1 [1] using results produced by the instument. The instrument was set to use the following parameters:

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CHEMISTRY PARAMETERS

TEST              [CR   ]
ASSAY CODE        [RATE-A  ]:[28]-[31]
SAMPLE VOLUME     [ 8][ 8]
R1 VOLUME         [250][100][NO ]
R2 VOLUME         [50 ][ 50][NO ]
WAVELENGTH        [570][505]
CALIB. METHOD     [LINEAR   ][0][0]
STD.(1) CONC.-POS.[  0.0]-[16]
STD.(2) CONC.-POS.[  2.0]-[17]
STD.(3) CONC.-POS.[     ]-[ 0]
STD.(4) CONC.-POS.[     ]-[ 0]
STD.(5) CONC.-POS.[     ]-[ 0]
STD.(6) CONC.-POS.[     ]-[ 0]
SD LIMIT          [  999]
DUPLICATE LIMIT   [32000]
SENSITIVITY LIMIT [    0]
ABS.LIMIT(INC/DEC)[32000][INCREASE]
PROZONE LIMIT     [     0][UPPER]
EXPECTED VALUE    [  20.0]-[ 400.0]
TECH. LIMIT       [  -0.1]-[ 400.0]
INSTRUMENT FACTOR [ 1.0]
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3 LOD/ULOL

3.1 Description of Methods

The limit of quantitation (LOQ) and limit of linearity (ULOL) are reverified at the 0.5 and 300 mg/dL levels, respectively. Quintuple analyses are performed at each point, and the resulting data are used to calculate the mean and sample standard deviation. The criteria for reverification are results within ±20% of the target values.

3.2 Summary of Statistical Data

	Control point	
	0.5 mg/dL	300.0 mg/dL
Mean	0.5	256.9
SD	0.04	4.09
CV%	9.3	1.6

3.3 Discussion

All results were within ±20% of the target values.

4 Carryover

The extent of carryover from samples at extreme out-of-range concentrations (X) of 0 and 1000 mg/dL to samples at the 2 mg/dL (D) decision point is evaluated annually.

4.1 Description of Methods

Carryover studies are performed using the method of Armbruster et al. [2] with the sequence

$$\mathrm{D}_1\,\mathrm{D}_2\,\mathrm{D}_3\,\mathrm{X}_1\,\mathrm{X}_2\,\mathrm{D}_4\,\mathrm{X}_3\,\mathrm{X}_4\,\mathrm{D}_5\,\mathrm{D}_6\,\mathrm{D}_7\,\mathrm{D}_8\,\mathrm{X}_5\,\mathrm{X}_6\,\mathrm{D}_9\,\mathrm{X}_7\,\mathrm{X}_8\,\mathrm{D}_{10}\,\mathrm{X}_9\,\mathrm{X}_{10}\,\mathrm{D}_{11}.$$

Percent carryover is evaluated as the percent difference in response of carryover candidate samples vs normal samples

$$100 \times \frac{[\mathrm{D}_4 + \mathrm{D}_5 + \mathrm{D}_9 + \mathrm{D}_{10} + \mathrm{D}_{11}] - [\mathrm{D}_2 + \mathrm{D}_3 + \mathrm{D}_6 + \mathrm{D}_7 + \mathrm{D}_8]}{[\mathrm{D}_2 + \mathrm{D}_3 + \mathrm{D}_6 + \mathrm{D}_7 + \mathrm{D}_8]}.$$

Two-sample t tests using pooled variances are used to compare the means between carryover candidates and the decision point,

$$\left.\begin{aligned} H_0&: \quad \mu_\mathrm{D} - \mu_\mathrm{X} = 0.4, \\ H_a&: \quad \mu_\mathrm{D} - \mu_\mathrm{X} < 0.4, \end{aligned}\right\} \quad \text{for blank carryover}$$

$$\left.\begin{aligned} H_0&: \quad \mu_\mathrm{X} - \mu_\mathrm{D} = 0.4, \\ H_a&: \quad \mu_\mathrm{X} - \mu_\mathrm{D} < 0.4, \end{aligned}\right\} \quad \text{for 1000 mg/dL carryover.}$$

The 0.4 mg/dL level is chosen for comparison as it constitutes 20% of the decision point value. Carryover is expected to bias results in the direction of the carryover concentration and the result is evaluated at a significance level $\alpha = 0.01$.

4.2 Analytical Results

	Carryover level	
	0 mg/dL	1000 mg/dL
Carryover %	2.0	-3.0
p-value	4.7×10^{-8}	3.3×10^{-8}

4.3 Discussion

Carryover from blank and 1000 mg/dL samples was determined to be a relatively insignificant factor. In both cases, the t tests support (i.e., $p \leq \alpha$) the conclusion that the difference in means between carryover candidates and decision point samples is less than 20% of the decision point cutoff. In addition, the apparent carryover followed a gradient opposite to the hypothetical bias that would be expected.

5 Specificity/Interference

Specificity studies are performed to assess the ability of the assay to discriminate the following substances from creatinine at the levels indicated:

Ascorbic acid	20, 50, and 100 mg/mL
Niacin	3.0, 7.5, and 15.0 mg/mL
Tropicana Apple Juice with Vitamin C	undiluted
Mountain Dew	undiluted
Visine Original	undiluted

5.1 Description of Methods

Units of concentration are converted to milligrams per deciliter for statistical calculations. Linear regression of the response, R, on concentration, C,

$$\mathrm{E}(R/C) = \beta_0 + \beta_1 C,$$

is performed for analytes that are tested at multiple concentrations. Percent cross-reactivity is calculated as

$$100 \times \frac{C_d}{C_a} = 100 \times \frac{20.0\beta_1}{20.0 - \beta_0},$$

where C_d/C_a is the ratio of the creatinine decision-point cutoff concentration, C_d, to the concentration of interfering analyte that is necessary to elicit an equivalent response, C_a.

5.2 Analytical Results

Undiluted Visine Original, Tropicana Apple Juice, and Mountain Dew produce responses of 0.1, 4.9, and 20.4 mg/dL, respectively. See Figure 1 for a graphical summary of the cross-reactivity data of the vitamins.

	Linear Regression Coefficients		
Analyte	β_0	β_1	*Cross-reactivity, %*
Ascorbic acid	1.80	2.44×10^{-3}	0.3
Niacin	-0.02	1.08×10^{-3}	0.1

5.3 Discussion

Both ascorbic acid and niacin possess a vanishingly small percentage of cross-reactivity at the 20 mg/dL decision point. Visine Original, Tropicana Apple Juice, and Mountain Dew produce responses below, between, and above both decision points, respectively.

6 Conclusions

1. The LOQ and ULOL were reverified at the 0.5 and 300 mg/dL levels, respectively.
2. No evidence for carryover was found for candidate samples at the 2.0 mg/dL decision point following blank or 1000 mg/dL samples.
3. The cross-reactivities of the vitamins ascorbic acid and niacin, and the assay response to several commercially available products with superficial similarity to urine was characterized.

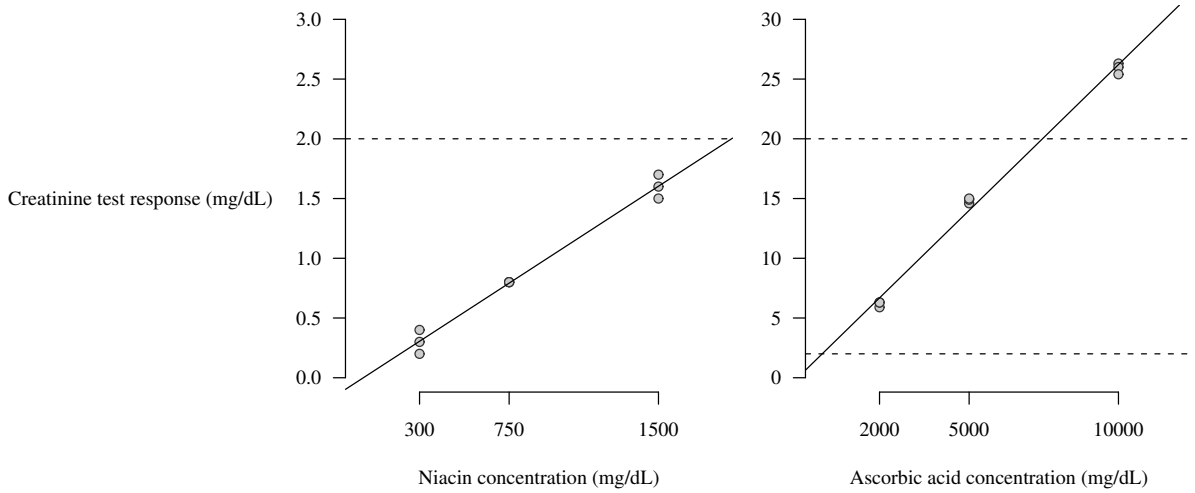

Figure 1: Cross-reactivities of niacin and ascorbic acid. Decision points are indicated by dashed lines.

This method meets requirements for reverification, and is valid for analysis of forensic urine samples under the Federal guidelines for a drug-free workplace.

Bibliography

(1) R Development Core Team, *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2008, ISBN 3-900051-07-0.

(2) Armbruster, D. A.; Schwarzhoff, R. H.; Hubster, E. C.; Liserio, M. K. Enzyme immunoassay, kinetic microparticle immunoassay, radioimmunoassay, and fluorescence polarization immunoassay compared for drugs-of-abuse screening. *Clin Chem* **1993 Oct**, *39*, 2137–2146.