Biomarker Qualification Letter of Intent (LOI) Template

Administrative Information

Requesting Organization

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If there is a prior, current, or planned submission to other regulatory agencies, list the agencies and dates as appropriate.

1. FDA (2008), EMA (2008) and PMDA (2010): Qualification of 5 biomarkers in rodent – Albumin (ALB), total protein, Kidney Injury Molecule-1 (KIM-1), Urinary Cystatin (CysC) and Clusterin (CLU).
2. FDA (August 20, 2014) and EMA (November 6, 2014) Letter of Support: Osteopontin (OPN) and Neutrophil Gelatinase-associated Lipocalin (NGAL) as kidney safety biomarkers in rodents.
3. PMDA Preliminary consultation meetings (January and February 2018); PMDA Briefing Book submitted in April 2018.
4. EMA: Parallel submission for Extended COU for kidney safety biomarkers.

Context of Use

Proposed Context of Use (COU) (limited to 500 characters)

Eight novel renal safety biomarkers are to be used together with conventional kidney biomarker monitoring, serum creatinine (sCr), in early clinical drug development trials to support conclusions as to whether a drug has caused a mild injury response in the renal tubules in healthy volunteers and patients with normal renal function.

Drug Development Need

Describe the drug development need that the biomarker is intended to address, including (if applicable) the proposed benefit over currently used biomarkers for similar COUs (limited to 1,500 characters).

The kidney is a common target organ of drug-induced toxicity. In nonclinical safety assessment studies, kidney injury associated with drug toxicity can be defined by histopathology. Drug-induced kidney injury (DIKI) is detected clinically by changes in standard laboratory tests for sCr, blood urea nitrogen (BUN) and Cystatin C (sCysC). These biomarkers are standard safety monitoring measures for kidney dysfunction, but are suboptimal when used for early signal detection of subtle injury in early clinical drug development settings. It is estimated that medically significant sCr elevation is not observed until greater than 50% of tubule function is compromised. In essence, standard indices of DIKI are lagging indicators of renal injury. The proposed 8 urinary kidney safety biomarkers have demonstrated greater sensitivity in detecting acute drug-induced tubular renal injury at earlier time points than current standard biomarkers in both humans and animals.

Biomarker Information

Biomarker name and description. If composite, please list the biomarker components.

Clusterin (CLU), product of CLU gene; urinary Cystatin C (CysC), product of CST3 gene; Kidney Injury Molecule-1 (KIM-1), product of TIM-1/HAVCR-1 gene; N-Acetyl-beta-D-Glucosaminidase (NAG), product of MGEA5 gene; Neutrophil Gelatinase-associated Lipocalin (NGAL), product of LCN2 gene; Osteopontin (OPN), product of SPP1 gene; albumin (ALB), product of ALB gene; and, Total Protein (gene N/A).

Type of Biomarker

- ✔ Molecular
- Histologic
- Other (please describe)
- Radiologic
- Physiologic characteristic

Biomarker Information

For molecular biomarkers, please provide a unique ID.

Scheme: UniProt (http://uniprot.org/)

ID: P10909 (CLU); P80188 (NGAL); P10451 (OPN)
O60502 (NAG); P02768 (ALB); Total Protein (No ID#)
P01034 (CysC); Q96D42 (KIM-1)

Matrix (e.g., blood) or modality (e.g., MRI): Urine

Primary biomarker category (see BEST Glossary): Please Select One

Describe the mechanistic rationale or biologic plausibility to support the biomarker and its associated COU (limited to 1,500 characters).

Five of the 8 biomarkers - ALB, total protein, KIM-1, CysC and CLU - have previously been qualified by the FDA, EMA and PMDA for use in rat studies conducted during drug development to support the safe conduct of clinical studies. Prospective studies have also been conducted to support the nonclinical and clinical utility of an additional 3 urinary kidney safety biomarkers: NAG, NGAL and OPN. These 8 biomarkers were selected based on a combination of factors, including association with acute kidney injury (biological plausibility [proximal tubular damage] and preclinical validation data); practicality; availability of assays; and clinical performance of the biomarker described in the literature. Furthermore, individual biomarkers in the panel were selected based on their location in the nephron in order to ensure the panel's response regardless of the site of nephrotoxic injury. Finally, the biomarker panel was deemed regulatory-ready based on literature reports and the individual biomarker's use in drug development clinical trials conducted by drug development companies. The 8 urinary biomarkers include tubular enzymes and other proteins that are induced in response to cellular injury and/or stress and inflammation, tubular proteins that are released into the urine after injury, and proteins that are normally filtered at the glomerulus and subsequently reabsorbed or metabolized by the tubules.

If biomarker is an index/scoring system, please provide information on how the index is derived (e.g., algorithm), the biologic rationale for inclusion of each of the components, the rationale for any differential weighting of the elements, and the meaning/interpretation of the index/score (limited to 1,500 characters).

No index scoring system. Biomarkers can be used as a panel or individually.

Biomarker Measurement Information

Provide a general description of what aspect of the biomarker is being measured and by what methodology (e.g., radiologic findings such as lesion number, specific measure of organ size, serum level of an analyte, change in the biomarker level relative to a reference such as baseline) (limited to 1,500 characters).

Biomarkers were measured in urine samples using rigorously validated commercially-available assays. The assays and associated validation documents have been reviewed by the FDA through the BQRT and deemed acceptable. See Attachment A, Table 1 for urine biomarker assay methods and manufacturers and Attachment A, Table 2 for proposed statistically and medically-significant thresholds used in prospective studies.

Is the biomarker test/assay currently available for public use? ◉ Yes ○ No

Indicate whether the biomarker test/assay is one or more of the following:

✔ Laboratory Developed Test (LDT)
✔ Research Use Only (RUO)
FDA Cleared/Approved. Provide 510(k)/PMA Number:

If the biomarker is qualified, will the test/assay be performed in a Clinical Laboratory Improvement Amendments (CLIA)–certified laboratory? ◉ Yes ○ No

Is the biomarker test currently under review by the Center for Devices and Radiological Health or the Center for Biologics Evaluation and Research?
○ Yes
◉ No
○ Don't Know

Is there a standard operating procedure (SOP) for sample collection and storage? ◉ Yes ○ No

Is there a laboratory SOP for the test/assay methodology? ◉ Yes ○ No

Biomarker Measurement Information

Describe the extent of analytical validation that has been performed (e.g., sensitivity, specificity, accuracy, and/or precision of the assay or method) (limited to 1,500 characters).

The assays used to support this qualification submission were developed in a Clinical Laboratory Improvement Amendments (CLIA) certified laboratory and validated as Laboratory Developed Tests (LDT). The protocols were complete and underwent a fit-for-purpose validation for their proposed context of use as biomarker tests (Lee 2006, FDA CDER 2013). Furthermore, all urine biomarker data for this proposed COU was generated at a single site laboratory, Pacific Biomarkers Incorporated (PBI). The PBI laboratory (645 Elliot Ave W., Suite 300, Seattle, WA, 98119; https://pacbio.com) is a CLIA certified and College of American Pathologists (CAP) accredited laboratory. Individual analyte assays were utilized for the quantification of the eight kidney translational safety biomarkers. Urinary CLU, CysC, KIM-1, NAG, NGAL, and OPN were measured with “For Research Use Only” assays using College of American Pathologists (CAP) compliant methods at PBI. ALB, total protein, and urinary creatinine (uCr) were measured using FDA-cleared assays according to manufacturer recommendations. The general assay validation performance characteristics are described in Attachment B, Table 1. Reported results from these assays we re-normalized to uCr concentrations as measured by the Roche Diagnostics enzymatic uCr assay. This uCr assay is standardized against ID-MS and the primary reference material used as a standard for the assay is the U.S. NIST SRM 914. See Attachment C, Table 1.

Additional Considerations for Radiographic Biomarkers

How has the method for image acquisition, analysis, and integration of the data been optimized? (Limited to 1,000 characters.)

Not Applicable

Does data currently exist to support the proposed cut point(s), if imaging results are not reported as a continuous variable?

○ Yes
○ No

Provide the name and version of the software package to be used for image acquisition and analysis (limited to 500 characters).

Not Applicable

Supporting Information

Please summarize existing preclinical or clinical data to support the biomarker in its COU (e.g., summaries of literature findings, previously conducted studies) (limited to 2,000 characters).

Nonclinical Phase - Cisplatin, aminoglycosides, and dozens of other nephrotoxicants were used to demonstrate the superiority of novel biomarkers over sCr for monitoring renal tubular injury by using histopathology as the gold standard. Outcomes: 1) Qualification of 7 biomarkers of drug-induced nephrotoxicity in rats (2008-2010) and 2) Letters of Support for OPN and NGAL (2014).
Clinical Learning Phase – 1) PSTC's Normal Healthy Volunteer (NHV) observational study, performed May 2011 - October 2012, established reference ranges for the 8 biomarkers of interest. Samples from 80 healthy volunteers were collected and analyzed to establish normal ranges. 2) In a Mesothelioma Cisplatin Study, novel urine biomarkers associated with DIKI were measured in samples from a study of mesothelioma cancer patients treated intraoperatively with high dose Cisplatin. This cohort of 58 patients underwent surgical resection (extrapleural pneumonectomy) for treatment of malignant pleural mesothelioma and received intraoperative intrathoracic Cisplatin, 250 mg/m2. These samples were collected between August 2008 and December 2010. Data from this study were used to determine medically-significant thresholds that indicate a patient has been exposed to a nephrotoxicant. Outcome: Submission of Limited COU Qualification Package to FDA (DDTBMQ000014).

Please summarize any planned studies to support the biomarker and COU. How will these studies address any current knowledge gaps? (Limited to 2,000 characters.)

Confirmatory Clinical Studies in patients currently using medications that have the potential to cause kidney injury: 1) Cisplatin Study and 2) Aminoglycoside Study. These studies are complete, but still undergoing analysis. In the Cisplatin Study, novel urine biomarkers associated with DIKI were measured in samples from head and neck squamous cell cancer patients treated with a single high dose of cisplatin (approximately 75 mg/m2). The Aminoglycoside Study was designed to evaluate novel biomarkers of aminoglycoside-induced kidney injury in adult cystic fibrosis (CF) patients, including those hospitalized for treatment of an acute pulmonary exacerbation and prescribed a beta-lactam and tobramycin at 5-10 mg/kg/day for up to 14 days; or hospitalized patients receiving a beta-lactam and fluoroquinolone antibiotic. CF outpatients not experiencing an exacerbation were included as controls. Data from these studies will allow project team statisticians and adjudication committee to confirm the medically significant thresholds for the biomarkers and demonstrate the biomarkers' greater diagnostic predictivity compared to serum creatinine.

Previous Regulatory Interactions

None

✔ Letter of Support (LOS) issued for this biomarker on date: 08/20/2014

Discussed in a Critical Path Innovation Meeting (CPIM) on date:

Previous FDA Qualification given to this biomarker with DDT Tracking Record Number:

Attachments

Please provide a list of publications relevant to this biomarker development proposal.

Optional* – If this biomarker development effort is part of a longer-term goal, please summarize your long-term objectives.*

Optional* – If you have other supporting information you would like to provide, please submit as attachment(s).

*Optional information will not be posted publicly.

Please refer to the Biomarker Qualification Contacts and Submitting Procedures for the mailing address and other important submission-related instructions. If you have any questions about submission procedures, please contact CDERBiomarkerQualificationProgram@fda.hhs.gov.

Table 1: Urine Biomarker Assay Method and Manufacturer used by PBI

Assay	OPN	KIM-1	CysC	Clusterin	NGAL	NAG	Total Protein	Albumin	Cr
Method	ELISA	ELISA	ELISA	ELISA	ELISA	Colori-metric	Turbidi-metric	Immuno-turbidimetric	Enzymatic
Manu-facturer	R&D Systems	R&D Systems	R&D Systems	R&D Systems	BioPorto Diagnostics	Roche Diagnostics	Roche Diagnostics	Roche Diagnostics	Roche Diagnostics

Table 2: Proposed Statistically and Medically Significant Thresholds for Use in the Prospective Studies

			Statistically Significant Thresholds			Medically Significant Thresholds		
Biomarker	**ULN**	**ULN FC from BL**	**T_{SS}**	**Specificity (%)**	**Sensitivity (%)**	**T_{MS}**	**Specificity (%)**	**Sensitivity (%)**
Clusterin	301 ng/mg uCr	2.3	FC > 2.6	94.9	95.0	FC > 6.0	77.1	60.0
OPN	2.10 µg/mg uCr	2.4	FC > 3.1	94.9	100.0	FC > 5.3	44.4	70.0
Albumin	30 µg/mg uCr	NA	> 1.0x ULN	97.5	100.0	> 10.0x ULN	97.1	40.0
Total Protein	0.20 mg/mg uCr	NA	> 1.0x ULN	96.2	100.0	>3.1x ULN	77.1	70.0
NAG	3.54 mU/mg uCr	2.1	FC > 3.9	100.0	100.0	FC > 8.7	65.7	55.0
KIM-1	1.19 ng/mg uCr	2.2	FC > 3.1	99.2	100.0	FC > 7.6	48.6	60.0
CysC	0.052 µg/mg uCr	2.2	FC > 2.2	96.6	95.0	FC > 4.5	71.4	70.0
NGAL	87.6 ng/mg uCr	3.2	FC> 3.5	95.9	95.0	FC > 9.6	59.5	60.0

ULNs = median of estimated ULNs based on Horn and Pesce method using PSTC NHV Study of Healthy Volunteers data; T_{SS} = Statistically significant threshold; T_{MS} = Medically significant threshold; Specificity/Sensitivity of Statistically Significant Thresholds based on Controls = NHV and Cases = mesothelioma patients with medically relevant increases in sCr; Specificity/Sensitivity of Medically Significant Thresholds based on Controls = mesothelioma patients without medically relevant increases in sCr and Cases = mesothelioma patients with medically relevant increases in sCr

ATTACHMENT B – Assay Validation Performance Characteristics

Table 1: Urine Biomarker Assay Method and Manufacturer

Assay	CLU	CysC	KIM-1	NGAL	NAG	OPN	Cr
Method	ELISA	ELISA	ELISA	ELISA	Colori-metric	ELISA	Enzymatic
Manu-facturer	R&D Systems	R&D Systems	R&D Systems	BioPorto Diagnostics	Roche Diagnostics	R&D Systems	Roche Diagnostics

Accuracy: The determination of accuracy was made using recovery experiments in which manufacturer supplied standards that consisted of recombinant human proteins or natural animal proteins were added ("spiked") to individual donor human urine samples in various concentrations to test the range of the standard curve. As the proteins being measured were all endogenous molecules, urine that had low concentrations of the analyte were used. These "spiked" samples were assayed and the measured values were compared to the calculated values. In all assays, the recovery was within the established criteria for recovery of 80-120%.

Precision: Precision was determined using standard CAP compliant methods. In general, within-run precision was evaluated using low, medium and high in-house control urine samples assayed 16 to 20 times in one analytical run. Between-run precision was evaluated using the same control samples used for within-run precision in three separate analytical runs.

Dilutional Linearity: Linearity was determined using standard CAP compliant methods. Verification of manufacturer's linearity range was performed using assay specific calibrator diluent. Mean recoveries for each sample were compared to target values using linear regression analysis.

Dynamic Range: PBI used the upper limit of linearity and the lower limit of sensitivity (usually lower limit of quantification [LLOQ]) for determining the dynamic range of each assay. When the sensitivity of the assay was below the lowest standard in an ELISA, for example, the lowest standard for the low end of the dynamic range was used. Limit of Detection (LOD) is generally a method for determining assay sensitivity that is based on the ability of an assay to distinguish the presence of analyte from instrument noise (no analyte present). Thus, LOD is lower than the LLOQ, which is the lowest level of analyte with a coefficient of variation (CV) of 20% or less. PBI used the more restrictive standard for sensitivity and this measure exceeds the LOD standard.

The upper limit of quantification (ULOQ) is defined as the highest level of analyte with a CV of 20% or less. PBI used the highest reference standard that met this criteria as the ULOQ. That number was then multiplied by the maximum dilution where assay linearity was still maintained to obtain the upper reportable limit (URL).

Interference: PBI conducted a series of studies to evaluate the potential interference of high concentrations of albumin, hemoglobin, and blood contamination on the measurement of urinary CLU, CysC, KIM-1, NAG, NGAL, OPN and uCr. Albumin (5 mg/mL), hemoglobin (~30 mg/dL), and blood (0.2%) did not interfere with the determination of urinary biomarker concentrations.

Albumin concentration greater than 3.0 mg/mL did interfere with the determination of urinary KIM-1. Thus, samples with greater than acceptable albumin will be diluted if KIM-1 levels are not already observed to be markedly elevated. In the original validation, linearity for KIM-1 was acceptable up to a 32-fold dilution. Furthermore, if a sample is observed to have moderate hemolysis, prior to the analysis for total protein, NGAL, NAG and KIM-1, these samples will be diluted until the measured hemoglobin concentration is 30 mg/dL or less. In the original validation, linearity for total protein, NGAL, NAG, and KIM-1 was acceptable up to a 54-fold, 64-fold, 3-fold, and 32-fold dilution, respectively.

Recovery: Recovery data were generated by PBI using either recombinant proteins or assay standards as outlined in the validation documents. Testing samples were prepared by the Merck Clinical Development Laboratory for all the biomarkers by adding known quantities of all proteins, except OPN, to urine. These samples were submitted to PBI for analysis to evaluate claimed recovery, precision and stability. The observed results were within or better than assay specifications stated in the vendor's validation documents.

Hook effect: To eliminate the possibility of a hook effect with values reported from the albumin assay, total protein concentration was evaluated prior to the analysis for albumin and samples with high values were appropriately diluted prior to performing the albumin assay. Hook effects may be dismissed by demonstrating linear dilution behavior throughout the expected maximum biological range of the analyte. In addition, hook effects are generally only of concern in homogeneous immunoassays. In a two-step ELISA (heterogeneous immunoassay), the hook effect is generally not of concern due to inclusion of wash steps to eliminate unbound excess analyte. This permits signal complexes to form at the maximum range of the assay yielding an over range condition (>ULOQ) rather than a falsely depressed value resulting from a hook effect (Ermens 2000).

Reagent Stability: PBI relied on individual manufacturer's claims of reagent stability.

Analyte Stability: Aliquots of urine were frozen at -70°C and used to examine stability (both 2-8°C storage and freeze/thaw cycle).

Bridging Studies: Due to a slight difference in the urine matrix used in the original assay validation and those collected in the studies to support qualification, PBI conducted a series of bridging studies to evaluate potential differences in the methods for measuring urinary CLU, CysC, KIM-1, NAG, NGAL, OPN and uCr. From these studies it was determined that the difference in urine matrices did not significantly affect the quantitation of the biomarkers and it was concluded that including a centrifugation step in urine sample processing is preferred to reduce the potential contribution of biomarker activity from cellular contamination.

Table 1: PSTC Kidney Safety Project Biomarker Qualification Assay Performance Summary

Biomarker	Albumin	Clusterin	Creatinine[e]	Creatinine	Cystatin-C	KIM-1	NAG	NGAL	Osteopontin	Protein (Total)
Platform	Roche Modular P	R&D ELISA	Modified Jaffé	Roche Modular P	R&D ELISA	R&D ELISA	Roche Modular P	BioPorto ELISA	R&D ELISA	Roche Modular P
Detection	Turbidometric	Colorimetric	Colorimetric	Colorimetric	Colorimetric	Colorimetric	Enzymatic colorimetric	Colorimetric	Colorimetric	Turbidometric
Precision WIR – L	2.6 %	10.5 %	0.7 %	1.0 %	3.9 %	8.3 %	4.3 %	5.4 %*	3.2 %	6.9 %
Precision WIR – M	1.4 %	6.3 %	1.0 %	2.4 %	3.0 %	1.1 %	2.5 %	8.8 %*	3.0 %	0.9 %
Precision WIR – H	1.1 %	5.8 %	0.8 %	0.7 %	4.3 %	5.3 %	1.9 %	8.7 %*	3.9 %	1.2 %
Precision BTR – L	4.6 %	14.5 %	2.2 %	0.9 %	1.3 %	11.3 %	5.6 %	-	12.5 %	8.5 %
Precision BTR – M	2.2 %	4.3 %	2.7 %	2.9 %	6.7 %	14.6 %	4.5 %	-	7.7 %	1.6 %
Precision BTR – H	1.5 %	4.7 %	2.2 %	1.5 %	5.8 %	15.8 %	2.5 %	-	10.0 %	1.4 %
Precision BTR – Mean (CV)	<5%	< 15%	-	< 3%	< 12%	< 16%	< 6%	< 7%	< 13%	<9%
Units	**mg/L**	**ng/mL**	**mg/dL**	**mg/dL**	**ng/mL**	**pg/mL**	**U/L**	**ng/mL**	**ng/mL**	**mg/dL**
Precision WIR – L Sample Value	5.64	16	-	49.2	14.2	169	0.86	4.2	807	6.9
Precision WIR – M Sample Value	31.53	105	-	97.4	27.2	579	2.41	20	1775	53.2
Precision WIR – H Sample Value	105.09	238	-	146.1	79.4	1161	9.9	44.5	4524	181.3
LLOQ	3.0	10 (Incl. PAD)	0.8	3.6	1.31	11.6	0.31	0.004 (Incl. PAD)	44 (Incl. PAD)	3.5
ULOQ	400	800	600	900	100	2000	55.25	100	8,800	200
Upper reportable limit	4,400	3,200	19,200	16,864	6,400	64,000	2,210 U/L	6,400	281,600	10,800
Recovery range	ND	90-107.5%	-	103.5-107.9%	83.8-104.2%	96.6-118%	99.1-104.5%	93.3-109.4%	97.9-101.5%	104.1-118.8%
Reference interval (normalized to uCr)	ND	35-383 ng/mg	-	40.0-278 mg/dL (M); 29.0-226 mg/dL (F)	0.014-0.058 µg/mg	<1.191 ng/mg	<0.78 U/mmol	<41.8 ng/mg	495-2029 ng/mg	1.3 – 10.1 mg/mg (x100)
Dilutional range	≤11-fold	≤4-fold (Pre-Diluted)	≤32-fold	≤32-fold	≤64-fold	≤32-fold	≤40-fold	≤64-fold (Pre-Diluted)	≤32-fold (Pre-Diluted)	≤54-fold
Dilutional linearity	±13.8%	±20%	±8.3	±4.9 %	±19.6%	±18.0%	±12.1%	ND	±8.3%	±20.3%
Procedural Dilution[d]	-	4	-	-	-	-	-	100	440	-
Interferences – no effect	-	-	Icterus <70 mg/dL Hb <966 mg/dL ascorbic acid <300 mg/L glucose <2000 mg/dL	Bilirubin <50 mg/dL Hb <1100 mg/dL Glucose <2100 mg/dL urobilinogen <40 mg/dL	-	-	-	-	Calcium <200 µg/mL	Icterus <36 mg/dL

Biomarker	Albumin	Clusterin		Creatinine[e]	Creatinine	Cystatin-C		KIM-1		NAG	NGAL		Osteopontin		Protein (Total)
				urobilinogen <40 mg/dL											
Interferences – False Negative	-	-		calcium dobesilate (Dexium) α-methyldopa	-	-		-		-	-		-		Many
Interferences – False Positive	-	-		-	cephalosporin	-		-		-	-		-		Many
Interferences - Quantitative measured as percentages	-	223 ng/ml H	110.8 ng/ml L	110.8 ng/ml		43.2 ng/ml H	18.1 ng/ml L	1.020 ng/ml H	0.358 ng/ml L	5.5 ng/ml	23.1 ng/ml H	11.4 ng/ml L	2325 ng/ml H	817n g/ml L	-
LLN/ULN	-	64.8 / 107.2		-		13.4 / 43.2		0.239/0.496		-	6.3/13.6		608/1106		-
Albumin	NA	7.5	21.9	-0.7		3.8	28.2	26.9	64.1	4.5	3.2	2.6	9.1	21.2	NA
Blood 1%	62.3	31.4	87.7	5.4		7.4	34.5	46.2	112.9	133.4	54.2	108.2	4.0	-12.7	94.9
Blood 0.8%	393.3	521.9	1392.7	-0.8		10.2	64.6	15.7	62.1	2.3	7.5	43.5	4.7	24.0	230.2
Blood 0.2%	94.3	103.5	218.8	0.2		-0.8	25.7	5.9	51.4	-0.2	3.0	7.4	2.6	21.5	54.3
HGB 0.8%	4.7	11.2	21	2.7		6.3	32.7	25.0	83.5	52.1	32.1	52	-1.5	1.6	31.9
HGB 0.2%	0.9	9.5	22.7	1.7		0.6	24.8	11.8	62.1	9.9	8.0	16.7	0.7	6.1	12.9
Stability	-	-		-	-	-		-		-	-		-		-
Ambient	N/A	N/A		-	1 day	Not stable for times tested		1 hour		N/A	3 hours		At least 2 hours		6 hours
2 – 8 C	N/A	N/A		-	7 day	1 day		3 days		5 days	3 days		6 days		2 days
Freeze/Thaw [b]	N/A	N/A		--	3	1		2		3	3		3		2

Biomarker	Albumin	Clusterin	Creatinine[e]	Creatinine	Cystatin-C	KIM-1	NAG	NGAL	Osteopontin	Protein (Total)
Long Term (-70C)	N/A	N/A	-	N/A	N/A	1 year c	1 year c	At least 3 months (PBI) 1 year c	N/A	N/A

BTR – Between Run
WIR – Within Run
PAD – Preanalytical Dilutions
ND – Not Determined
a information is for urine with no preservatives added
b *The stability method reflects the number of Freeze/Thaw cycles after initial freeze*
c *Han WK et al. Urinary biomarkers in early detection of acute kidney injury after cardiac surgery. Clin J Am Soc Nephrol 4:873-882, 2009.*
d Procedural dilution mandated by the manufacturer's protocol, performed on every sample including standards and controls, thus factor out.
LLN and ULN – Upper Limit of Normal and Lower Limit of Normal as determined in this study
NA – Not Applicable

Based on the previously submitted PBI document, 0.8% washed blood is reported as 1.2 g/L Hbg. From Tietz, Mean Corpuscular Hemoglobin (MCH) 95% normal range for adults (male and female) is 31±4 pg Hbg per erythrocyte. Thus,
0.8 % washed blood = (1.2 g/L Hbg) / ($3.1 \cdot 10^{-12}$ g Hbg/Ery) = $0.387 \cdot 10^{6}$ Ery/µL
0.2 % washed blood = (0.3 g/L Hbg) / ($3.1 \cdot 10^{-12}$ g Hbg/Ery) = $0.096 \cdot 10^{6}$ Ery/µL

e Two creatinine columns are listed due to two creatinine assay platforms being employed

ATTACHMENT D – Publications Relevant to Proposed Biomarkers

Agarwal, Rajiv. 2004. "Statin Induced Proteinuria: Renal Injury or Renoprotection?" *Journal of the American Society of Nephrology: JASN* 15 (9): 2502–3. doi:10.1097/01.ASN.0000143720.71748.79.

Amin, Rupesh P., Alison E. Vickers, Frank Sistare, Karol L. Thompson, Richard J. Roman, Michael Lawton, Jeffrey Kramer, et al. 2004. "Identification of Putative Gene Based Markers of Renal Toxicity." *Environmental Health Perspectives* 112 (4): 465–79.

Ariza, Xavier, Elsa Solà, Chiara Elia, Rogelio Barreto, Rebeca Moreira, Manuel Morales-Ruiz, Isabel Graupera, et al. 2015. "Analysis of a Urinary Biomarker Panel for Clinical Outcomes Assessment in Cirrhosis." *PloS One* 10 (6): e0128145. doi:10.1371/journal.pone.0128145.

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