DETECTION OF INFLAMMATORY BIOMARKERS IN URINE USING A DRIED, SHELF STABLE TRANSPORT DEVICE, VIVEST™

KIDNEYWEEK 2015 San Diego, CA • Nov 3-8

Poster ID
FR-PO290

AM McClernon[1], TW Murray[2], DR McClernon[1]
[1]bioMONTR® Labs, Research Triangle Park, NC
[2]ViveBio LLC, Alpharetta, GA

Introduction

Current medical practice assesses the health of the implanted kidney by monitoring non-specific signals (serum creatinine) within the body. Unfortunately, these signals can only be measured after the process of rejection has started and the gold standard diagnostic method is an invasive kidney biopsy, which carries risk.

Recent studies of non-invasive biomarkers (CXCL9) have shown utility (CTOT-01) in identifying subsets of patients at risk for acute rejection. A urine based test for post surgical evaluations will enable doctors to pinpoint the rejection cycle and expedite the appropriate therapeutic intervention.

Here-in we describe detection of CXCL9/MIG and CXCL10/IP-10 in urine spectrophotometrically, in combination with a novel collection device ViveST, eliminating the need for cold chain transport and storage of the urine specimen.

Methods

"Clean catch" urine samples from healthy donors were collected, placed on ice within one hour, transferred to 15 mL conical tubes and centrifuged (4°C) @ 2,000 x g for 30 minutes. The supernatant from each was decanted into clean 15 mL conical tubes and diluted 1:1 with proprietary sample diluent buffer. Recombinant Human CXCL9/MIG and CXCL10/IP-10 (R&D Systems) was reconstituted using molecular grade water to concentrations of 10,000 pg/µL and serially diluted.

The prepared urine was spiked with diluted recombinant human MIG or IP-10. 1 mL aliquots of each were loaded onto ViveST and dried overnight. The following day, ViveST samples were reconstituted with 1 mL of molecular grade water and analyzed concurrently with frozen aliquots using quantitative sandwich enzyme immunoassay techniques specific for MIG or IP-10. Seven levels of calibration standard were run concurrently to allow for quantitative analysis.

Linear regression analysis was used to calculate MIG and IP-10 concentrations in each sample.

Results

For all concentrations of CXCL10/IP-10, the ViveST samples yielded IP-10 concentrations similar to the corresponding frozen aliquots (see Figure 1 and Table 1). For all concentrations of CXCL9/MIG, the ViveST samples yielded concentrations similar to the corresponding frozen aliquots (data not shown).

For all concentrations of CXCL10/IP-10 and CXCL9/MIG, replicate aliquots on ViveST gave similar results compared to the frozen aliquots (see Table 1).

Results

Figure 1. Detection of CXCL10/IP-10 in Urine_Frozen & ViveST Samples

Position	1	2	3	4	5	6
A	Neg Ctrl	ViveST_Level 1 (Male)	IP10_ViveST Control	ViveST_Level 1 (Male)	Frozen_Level 1 (Male)	Frozen_Level 1 (Male)
B	1,000 pg/mL	ViveST_Level 2 (Male)	IP10_Frozen Control	ViveST_Level 2 (Male)	Frozen_Level 2 (Male)	Frozen_Level 2 (Male)
C	500 pg/mL	ViveST_Level 3 (Male)	Negative Urine	ViveST_Level 3 (Male)	Frozen_Level 3 (Male)	Frozen_Level 3 (Male)
D	250 pg/mL	ViveST_Level 4 (Male)		ViveST_Level 4 (Male)	Frozen_Level 4 (Male)	Frozen_Level 4 (Male)
E	125 pg/mL	ViveST_Level 1 (Female)		ViveST_Level 1 (Female)	Frozen_Level 1 (Female)	Frozen_Level 1 (Female)
F	62.5 pg/mL	ViveST_Level 2 (Female)		ViveST_Level 2 (Female)	Frozen_Level 2 (Female)	Frozen_Level 2 (Female)
G	31.2 pg/mL	ViveST_Level 3 (Female)		ViveST_Level 3 (Female)	Frozen_Level 3 (Female)	Frozen_Level 3 (Female)
H	15.6 pg/mL	ViveST_Level 4 (Female)		ViveST_Level 4 (Female)	Frozen_Level 4 (Female)	Frozen_Level 4 (Female)

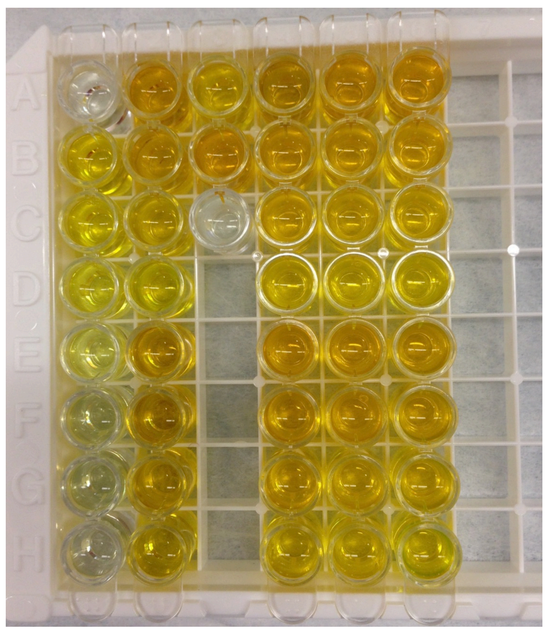

Table 1. Concentration of CXCL9/MIG and CXCL10/IP-10 in Urine_Frozen & ViveST Samples

	Concentration of MIG (pg/uL)			Concentration of IP-10 (pg/uL)			
				Male Urine (pg/uL)		Female Urine	
	ViveST (n = 3)	Frozen (n = 1)		ViveST (n=2)	Frozen (n=2)	ViveST (n=2)	Frozen (n=2)
Level 1	117	98		>1572	>1572	>1572	>1572
Level 2	67	53		>1572	>1572	>1572	>1572
Level 3	34	27		1534	1472	1541	1501
Level 4	14	11		1096	1029	1157	1042
Level 5	5	2		Level 5 - Level 7 not included in this experiment			
Level 6	0	2					
Level 7	0	0					

Conclusions

- MIG and IP-10 can be detected in urine as a biomarker to asses risk of transplant rejection.
- ViveST can be used as an ambient storage and transport device eliminating need for cold chain storage.
- Additional studies are needed to assess detection of biomarkers at therapeutic relevant concentrations.
- Additional studies are needed for evaluation of additional biomarkers that may be present in urine.

Send correspondence to:
Daniel McClernon
dmcclernon@biomontr.com

bioMONTR® Labs