TEST REPORT

Lishtot TestDrop™ Pro – Performance Testing for Detection of Protein Matter in Water

Date: January 5, 2018

Customer: Lishtot, Ltd.

Testing Conducted by: Alexander Belgovskiy, B.Sc., M.Sc.
Report Compiled by: Alexander Belgovskiy, B.Sc., M.Sc.

Report Approved by: Aimee Kestranek
Title: VP, Analytical Services

Signed by:

Alexander Belgovskiy

Alexander Belgovskiy

Aimee N. Kestranek

Aimee Kestranek

Analiza, Inc.
3615 Superior Ave. Suite 4407B
Cleveland, OH 44114
Tel/fax: 216-432-9050
www.analiza.com

1. Table of Contents

2. General Information

2.1 Purpose

Report data and analysis of findings from a study conducted to quantitatively determine the detection performance of protein matter in the range of 5 – 1000 ng/ml (numerically equivalent to 5 – 1000 parts per billion) dissolved in pure water using Lishtot TestDrop™ Pro device.

2.2 Scope

The study is designed to demonstrate performance of Lishtot TestDrop™ Pro device in controlled conditions. Protein matter was represented by a common protein, Bovine Serum Albumin (BSA) and pure water was represented by HPLC grade water. Performance with other proteins or organic substances and in presence of other contaminants in water may differ.

2.3 Definitions, Acronyms and Abbreviations

BSA: Bovine Serum Albumin

BW: Bottled Water

HPLC: High Performance Liquid Chromatography

mg/ml: concentration unit, milligram per milliliter

ng/ml: concentration unit, nanogram per milliliter

SDS: Safety Data Sheet

TW: Tap Water

2.4 Background

The Lishtot TestDrop™ Pro device is designed to detect impurities in water. In this report we focus on the ability of the device to detect the presence of organic matter in water.

The device operation is based on measurement and analysis of the electrostatic field in the vicinity of a container with tested water. The device reports findings using blue or red indicator lights, and can also transfer the raw time-based measurements of the electrostatic field via a Bluetooth connection with cell phone or a computer for further processing and reporting.

2.5 Point of Contact

Aimee Kestranek; Analiza, Inc., Cleveland, OH

3. Testing Schedule

Test samples were prepared and tested at the laboratories of Analiza, Inc. in Cleveland, Ohio, conducted between 12/18/2017 and 1/5/2018.

4. Testing Characteristics

4.1 Materials and Equipment

- *Lishtot TestDrop Pro device:* Device ID: 00:A0:50:80:49:95
- *Test cup*: "Disposoware" 5 oz. polypropylene cup
- *Water*: Water, HPLC grade, "OmniSolv", EMD Millipore Corp., CAS-No: 7732-18-5, lot 57297, expiration date: 2018/10/31.
- *Protein*: Bovine Serum Albumin (heat shock fraction), Sigma, P/N A-7906, lot 113F-0123.
- *Analytical balances:* Mettler Toledo, AB204-S/FACT.
- *Pipette:* BioHit Single Channel Adjustable Volume Mechanical Pipette m1000
- *Graduated Glass Cylinders:* Pyrex No. 3042 (1000 ml), No. 3046 (50 ml), No. 3046 (25 ml).
- *Glass bottles:* Pyrex No. 1395, 1000 ml; Wheaton, 500 ml; VWR, Cat. No. 89000-236, 200 ml.

4.2 Safety Considerations

The following safety practices were followed during testing:

- Sturdy closed-toe, water-proof shoes must be worn in the laboratory at all times.
- No food, beverages, cosmetics or medications should be consumed or used within the laboratory.
- Review relevant Safety Data Sheets (SDS) for materials being used.
- Be aware of location and use of eye wash stations and emergency showers.

Note: The Lishtot TestDrop Pro device must be used with bare hands (no protective gloves worn), since protective gloves may interfere with electrostatic field near the test object, which may lead to invalid test results.

4.3 Test Design

The following concentrations of BSA protein in HPLC grade water were chosen for testing: 1000, 100, 50, 35, 25, 10, and 5 ng/ml. Pure HPLC grade water was designated as negative control. Each protein solution and the negative control were tested consecutively 10 times (which matches the capacity of data storage of the device). Preparation of solutions and testing were performed on the same day.

4.4 Procedure

All solutions were prepared using glass labware. The glass containers for solutions were washed inside with a bottle brush using dish detergent (with no hand moisturizers), then washed with hot tap water until no foam is present, then rinsed with hot tap water minimum 12 times (partially filling and emptying the container), and then rinsed with deionized water minimum of 5 times (partially filling and emptying the container), and then finally rinsed with HPLC grade water 2 times.

Glass cylinders used to handle protein solutions were first rinsed with the same solution and only then used to sample the solution. The manual pipette tip rinsed with the test solution prior to transferring the solution by reverse pipetting technique. This was done to minimize the impact of possible adsorption of protein on the surface of the pipette, especially consequential at low protein concentrations.

Stock solution (200 ml) of BSA protein, 1 mg/ml, was prepared. From that stock solution, 1000 ml of 1000 ng/ml solution was prepared. From that solution, 500 ml of each of the following solutions in HPLC grade water were prepared: 100, 50, 35, 25, 10 and 5 ng/ml, according to the following procedure:

1. 200 ml of stock solution of BSA protein, 1 mg/ml, were prepared by dissolving of 200 mg of dry protein in 200 ml of HPLC grade water, in a clean glass bottle.
2. 1000 ml of 1000 ng/ml solution were prepared in a clean bottle by mixing 1 ml of the stock solution (1) and approximately 999 ml of HPLC grade water.
3. 500 ml of 100 ng/ml solution were prepared in a clean bottle by mixing 50 ml of the 1000 ng/ml solution and 450 ml of HPLC grade water.
4. 500 ml of 50 ng/ml solution were prepared in a clean bottle by mixing 25 ml of the 1000 ng/ml solution and 475 ml of HPLC grade water.
5. 500 ml of 35 ng/ml solution were prepared in a clean bottle by mixing 17.5 ml of the 1000 ng/ml solution and approximately 482.5 ml of HPLC grade water.
6. 500 ml of 25 ng/ml solution were prepared in a clean bottle by mixing 12.5 ml of the 1000 ng/ml solution and approximately 487.5 ml of HPLC grade water.

7. 500 ml of 10 ng/ml solution were prepared in a clean bottle by mixing 5 ml of the 1000 ng/ml solution and 495 ml of HPLC grade water.
8. 500 ml of 5 ng/ml solution were prepared in a clean bottle by mixing 2.5 ml of the 1000 ng/ml solution and approximately 497.5 ml of HPLC grade water.

All solutions were carefully and gently mixed before further dilutions and before performing tests.

Preparation of all solutions (including the stock solution) was performed on testing day. Testing was performed over two non-consecutive days.

Before testing, the negative control (i.e. pure HPLC water) was put in a bottle cleaned in the same way as the bottles for other test solutions. This was done to make sure no detectable contaminants are left on the bottle surface after labware cleaning procedure.

Each individual test was performed as follows:

1. A clean 5 oz. polypropylene test cup was placed on a non-metalic (Formica®) laboratory bench.
2. Approximately 80 ml of test solution were poured into the cup.
3. The cup was gently swirled for about 5 seconds, avoiding spills.
4. The Lishtot TestDrop Pro device was positioned at the side of the cup, a few inches away.
5. The desired button (TW or BW) on the device was pressed (a yellow LED starts blinking at the tip of the device).
6. The device was moved towards the cup in a single motion, touching the cup about 2 to 5 mm above the liquid level, while still holding a button on the device.
7. Upon touching the cup, and allowing for one extra blink of the yellow LED, the button was released.

To repeat the test with the same sample, steps (3) to (7) were repeated.

A minimum of 5 seconds were allowed between the end of the test and the beginning of the next test.

When the red light on the device indicated that the battery is weak, the battery was replaced with a fresh one. After replacing the battery, the “High” setting was reset, with positive confirmation by the message box.

Each test was repeated 10 consecutive times with the same sample and cup combination. After 10 consecutive tests, the data was transferred from the device to a smartphone (Samsung Galaxy S7) using Lishtot Android app via Bluetooth interface (the device buffer holds data for the last 10 tests). Series of tests of negative control sample were interspersed with series of tests of protein solutions.

5. Results

5.1 Data

The following data were transferred from the TestDrop™ Pro device to the smartphone and the data server:

5.1.1 Table 1. Groups of repeat tests for the same solution.

Test #	Time Stamp	Button	BSA Concentration, ug/ml*	Sensitivity Setting	LED Color
121	12/18/2017 13:48:08	BW	1000	High	RED
122	12/18/2017 13:48:43	TW	1000	High	RED
123	12/18/2017 13:48:57	BW	1000	High	RED
124	12/18/2017 13:46:15	TW	1000	High	RED
125	12/18/2017 13:46:24	TW	1000	High	RED
126	12/18/2017 13:46:41	TW	1000	High	RED
127	12/18/2017 13:46:49	TW	1000	High	RED
128	12/18/2017 13:46:56	TW	1000	High	RED
129	12/18/2017 13:47:04	TW	1000	High	RED
130	12/18/2017 13:47:12	TW	1000	High	RED
135	12/18/2017 15:26:27	TW	100	High	RED
136	12/18/2017 15:26:52	TW	100	High	RED
137	12/18/2017 15:27:07	TW	100	High	RED
138	12/18/2017 15:27:37	TW	100	High	RED
139	12/18/2017 15:27:46	TW	100	High	RED
140	12/18/2017 15:28:00	TW	100	High	RED
141	12/18/2017 15:28:13	TW	100	High	RED
142	12/18/2017 15:28:26	TW	100	High	RED
143	12/18/2017 15:28:39	TW	100	High	RED
144	12/18/2017 15:28:52	TW	100	High	RED
145	12/18/2017 16:25:44	TW	50	High	RED
146	12/18/2017 16:26:00	TW	50	High	RED
147	12/18/2017 16:26:23	TW	50	High	RED
148	12/18/2017 16:27:23	TW	50	High	RED
149	12/18/2017 16:27:37	TW	50	High	RED
150	12/18/2017 16:27:54	TW	50	High	RED
151	12/18/2017 16:28:06	TW	50	High	RED
152	12/18/2017 16:28:25	BW	50	High	RED
153	12/18/2017 16:28:41	BW	50	High	RED
154	12/18/2017 16:28:58	BW	50	High	RED
175	12/18/2017 18:04:01	TW	0 (Negative Control)	High	BLUE
176	12/18/2017 18:01:46	TW	0 (Negative Control)	High	BLUE

Test #	Time Stamp	Button	BSA Concentration, ug/ml*	Sensitivity Setting	LED Color
177	12/18/2017 18:01:57	TW	0 (Negative Control)	High	BLUE
178	12/18/2017 18:02:12	TW	0 (Negative Control)	High	BLUE
179	12/18/2017 18:02:28	TW	0 (Negative Control)	High	BLUE
180	12/18/2017 18:02:47	TW	0 (Negative Control)	High	BLUE
181	12/18/2017 18:03:03	TW	0 (Negative Control)	High	BLUE
182	12/18/2017 18:03:19	TW	0 (Negative Control)	High	BLUE
183	12/18/2017 18:03:33	TW	0 (Negative Control)	High	BLUE
184	12/18/2017 18:03:47	TW	0 (Negative Control)	High	BLUE
495	1/3/2018 15:51:02	TW	35	High	RED
496	1/3/2018 15:51:17	TW	35	High	RED
497	1/3/2018 15:51:31	TW	35	High	RED
498	1/3/2018 15:51:44	TW	35	High	RED
499	1/3/2018 15:51:56	TW	35	High	RED
500	1/3/2018 15:52:08	TW	35	High	RED
501	1/3/2018 15:52:22	TW	35	High	RED
502	1/3/2018 15:52:35	TW	35	High	RED
503	1/3/2018 15:52:47	TW	35	High	RED
504	1/3/2018 15:52:59	TW	35	High	RED
505	1/3/2018 16:13:15	TW	25	High	RED
506	1/3/2018 16:13:29	TW	25	High	RED
507	1/3/2018 16:13:44	TW	25	High	RED
508	1/3/2018 16:13:57	TW	25	High	RED
509	1/3/2018 16:14:12	TW	25	High	RED
510	1/3/2018 16:14:24	TW	25	High	RED
511	1/3/2018 16:14:38	TW	25	High	RED
512	1/3/2018 16:14:50	TW	25	High	RED
513	1/3/2018 16:15:04	TW	25	High	RED
514	1/3/2018 16:15:18	TW	25	High	RED
515	1/3/2018 16:30:03	TW	0 (Negative Control)	High	BLUE
516	1/3/2018 16:30:30	TW	0 (Negative Control)	High	BLUE
517	1/3/2018 16:30:42	TW	0 (Negative Control)	High	BLUE
518	1/3/2018 16:30:54	TW	0 (Negative Control)	High	BLUE
519	1/3/2018 16:31:05	TW	0 (Negative Control)	High	BLUE
520	1/3/2018 16:31:18	TW	0 (Negative Control)	High	BLUE
521	1/3/2018 16:31:29	TW	0 (Negative Control)	High	BLUE
522	1/3/2018 16:31:41	TW	0 (Negative Control)	High	BLUE
523	1/3/2018 16:31:52	TW	0 (Negative Control)	High	BLUE
524	1/3/2018 16:32:29	TW	0 (Negative Control)	High	BLUE
535	1/3/2018 17:15:19	TW	10	High	RED
536	1/3/2018 17:15:42	TW	10	High	RED
537	1/3/2018 17:15:56	TW	10	High	RED
538	1/3/2018 17:16:12	TW	10	High	RED
539	1/3/2018 17:16:29	TW	10	High	RED
540	1/3/2018 17:16:45	TW	10	High	RED
541	1/3/2018 17:17:00	TW	10	High	RED
542	1/3/2018 17:17:15	TW	10	High	RED
543	1/3/2018 17:17:29	TW	10	High	RED
544	1/3/2018 17:17:44	TW	10	High	RED

Test #	Time Stamp	Button	BSA Concentration, ug/ml*	Sensitivity Setting	LED Color
545	1/3/2018 17:57:22	TW	5	High	RED
546	1/3/2018 17:57:36	TW	5	High	RED
547	1/3/2018 17:57:50	TW	5	High	RED
548	1/3/2018 17:58:03	TW	5	High	RED
549	1/3/2018 17:58:15	TW	5	High	RED
550	1/3/2018 17:58:27	TW	5	High	RED
551	1/3/2018 17:58:39	TW	5	High	RED
552	1/3/2018 17:58:51	TW	5	High	RED
553	1/3/2018 17:59:02	TW	5	High	RED
554	1/3/2018 17:59:16	TW	5	High	RED

*It is noted that since some protein matter may be adsorbed at the inner surface of the glass labware, the nominal reported concentration level should be viewed as upper limit; namely, the actual solution concentration could be lower than the calculated nominal level, particularly at lower concentrations.

5.1.2 Table 2. Interspersed tests of protein test solution and negative control.

Test #	Time Stamp	Button	BSA Concentration, ug/ml	Sensitivity Setting	LED Color
525	1/3/2018 16:40:22	TW	25	High	RED
526	1/3/2018 16:40:48	TW	0 (Negative Control)	High	BLUE
527	1/3/2018 16:41:07	TW	25	High	RED
528	1/3/2018 16:41:25	TW	0 (Negative Control)	High	BLUE
529	1/3/2018 16:41:43	TW	25	High	RED
530	1/3/2018 16:41:59	TW	0 (Negative Control)	High	BLUE
531	1/3/2018 16:42:16	TW	0 (Negative Control)	High	BLUE
532	1/3/2018 16:42:34	TW	25	High	RED
533	1/3/2018 16:42:47	TW	25	High	RED
534	1/3/2018 16:43:02	TW	0 (Negative Control)	High	BLUE

Red LED color means that the device positively detects presence of protein in the liquid.

Blue LED color means that the device did not detect any protein in the liquid.

6. Conclusions

Based on obtained data, the Lishtot TestDrop™ Pro device was able to consistently detect presence of protein matter (represented by BSA) in pure water (represented by HPLC grade water) at concentration as low as 5 ng/ml (or numerically equivalent to 5 parts per billion, ppb). The device was also able to consistently report the absence of protein matter in pure water.

It is noted that since some protein matter may be adsorbed at the inner surface of the glass labware, the nominal reported concentration level should be viewed as upper limit; namely, the actual solution concentration as tested could be lower than the calculated nominal level, particularly at lower concentrations.

Revision History			
Revision Level	Effective Date	Initiator	DCO Number
A	1-5-2018		DCO-00001
Section Number	Description and Justification of Changes		
All	Initial Release		

Legal Disclaimer

This report is generated in connection with specific testing protocol, test articles, and testing methodologies. The results and conclusion provided herein are limited to the study conducted and are not predictive of operational behavior and performance with different testing protocols, test articles, or test methodologies. No expressed or implied warranty is provided for any fitness for any purpose of the test articles.