ORIGINAL ARTICLE

Analysis of a Comparability Test between LX Detergent Cleaning Solution and OC Detergent Cleaning Solution Using OC Sensor PLEDIA

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OC Sensor PLEDIA를 이용한 LX Detergent Cleaning Solution과 OC Detergent Cleaning Solution의 동등성 평가

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ABSTRACT

This study aimed at comparing the performance of imported LX detergent cleaning solution (LX-CS) and the self-manufactured OC cleaning solution (OC-CS), based on functional and quantitative analysis. The functional analysis was carried out using apparent diffusion coefficient (ADC) values. For quantitative analysis, precision, linearity, and carry-over rates were measured with commercial control materials according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Using OC-Sensor PLEDIA (Eiken Chemical, Japan), the ADC value of all cuvettes satisfied the acceptance criteria. For quantitative analysis, precision was less than 5.0% for the two products, and carry-over rates were less than $\pm 1.00\%$. The linearity slopes and r^2 values were 1.0017 and 0.9982 in the LX-CS, and 0.9924 and 0.9996 in the OC-CS, respectively. The correlation coefficient (r) was found to be 0.9997. Also, the percent difference in correlation with 40 artificial-stool specimens was less than 10% and the p -value was less than 0.1. The result of standard deviation ratio ($D: \pm 1$ SD ratio) was similar for both products. In conclusion, the functional and quantitative analyses of the two products were compared and showed similar results. In the future, the self-manufactured OC-CS will be able to provide a much more stable and faster supply than the imported LX-CS.

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INTRODUCTION

In modern society, cancer incidence and mortality continue to rise due to the aging population, and the incidence of colorectal cancer in Koreans is significantly increasing due to westernized diet. In 2014,

colorectal cancer ranked 3rd among all the cancers. In Korea, colorectal cancer has been included in the targets of the national cancer screening project since 2004, and according to the colorectal cancer screening program recommendations, a fecal occult blood test (FOBT) should be performed every year or two in asymptomatic adults aged 45 to 80 as a colorectal cancer screening test [1]. In the case of colorectal cancer, since the stage when the cancer is diagnosed is an important factor that affects the survival rate, thus

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colorectal cancer screening test plays a huge role in the early detection and elimination of cancer [2].

There are qualitative and quantitative methods of the fecal occult blood test. In the chemical method (Guaiac method), the colorimetric reaction with pseudoperoxidase of hemoglobin is used, so it has the advantage of being inexpensive and easy to use. However, it has a high frequency of false positives and false negatives, and dietary restrictions need to be imposed, and its sensitivity and specificity are so low that even upper gastrointestinal bleeding is shown as positive. Thus, it can increase the false-positive rate in colorectal cancer screening [3]. For this reason, the guaiac method is inappropriate for use in the national cancer screening program because dietary restrictions and medication intake are required before diagnostic testing. In the fecal immunological test (FIT method), a globin antigen of human hemoglobin and a reaction of a single or polyclonal antibody to it were used to enhance the sensitivity and specificity of the test [4]. There are other methods: immunochromatography assay (ICA), a qualitative method using an aggregation method, and a quantitative method using a turbidity measurement method or a colorimetric method. Compared to chemical methods, immunological qualitative methods are easier to use for screening colorectal cancer because they have no restrictions on diet or medications, and only detect lower gastrointestinal bleeding. Therefore, immunological tests are used more often in the national cancer screening program [5, 6].

Recently, more than 10 types of the fully automated quantitative analyzer for the fecal occult blood test have been introduced to diagnostic laboratory medicine departments in Korea [7]. The OC Sensor Series diagnostic analyzer, which has a high domestic market share of external quality control standards managed by external quality assessment agencies in Korea, is widely used as a fecal blood screening test for early diagnosis of colorectal cancer. The OC Sensor PLEDIA is divided into the main body for detecting occult blood in feces, data management system (DMS),

main reagent, buffer solution, and other consumables [8].

Currently, the main reagents used in the OC sensor series are measured with immunoturbidimetry (IT) at 660 nm using polyethylene latex particles, and the buffer solutions are to maintain an appropriate pH and to facilitate antigen-antibody binding. Besides, the consumable cleaning solutions are used for analyzer instrument maintenance and cleaning and removal of contaminants in flexible plastic nozzle tubing. The purpose of this study sought to compare the performance of functional and quantitative analysis of the imported LX detergent cleaning solution (LX-CS) and the self-manufactured detergent for rapid product supply and stable storage through localization of cleaning solution of the OC sensor series.

MATERIALS AND METHODS

1. Sample collection and preparation

For evaluation, OC DIANA Latex reagents, buffer solutions, quality controls, and calibrators were used for OC Sensor PLEDIA. The imported LX-CS and self-manufactured OC-CS were verified in the same method and volume under the same condition.

The artificial-stool specimens used in the current work were sampled by homogeneously mixing the surface of the specimen using a sampling bottle, which was dipped in 5~6 places, and inspected after collection. The substance in the sampling bottle corresponds to artificial-stool specimens of about 10 mg and it was strongly shaken so that it was enough homogeneously suspended in 2 mL buffer solution. After being left at room temperature, it was subjected to the test. The pre-processed sampling bottle was mounted on the specific rack and an automated analyzer produces the inspection results. And then, the main reagent and the internal quality control substance were sufficiently warmed to room temperature and tested, and calibration was conducted based on the criteria appropriate to the conditions. The reference value was 100 ng/mL, which was equivalent to 20 µg of

hemoglobin in 1 g of feces. The analytical measurable range (AMR) of OC sensor PLEDIA was 50~1000 ng/mL, and if over 1000 ng/mL, it was recorded as over-range (OR).

2. Preparation of artificial-stools

Artificial-stools was a substance that replaces the patient's feces and was prepared by dividing the basic raw material powder and NOC buffer. The base raw powder was prepared by mixing bean flour, weak flour, strong flour, and sucrose. The hemoglobin (Hb) powder (Sigma Aldrich, Korea) was accurately weighed and mixed after dissolved in the NOC buffer solution. The NOC buffer solution used in this study was prepared as fresh as possible with distilled water, HEPES, NaCl, CyDTA, NaN_3 , BSA, and its pH is adjusted to 7.0 using 4N NaOH. After repeatedly measuring the prepared artificial-stools, they were prepared for each concentration and replaced with the evaluation materials (Table 1).

3. Preparation of products

The imported LX-CS using LC-MS (Liquid Chromatography-Mass Spectrometry) contained 5~6% of sodium hypochlorite (NaClO) and 0.5~0.8% of sodium hydroxide (NaOH) as a result of component analysis. The component analysis of the self-manufactured OC-CS showed that it contained 5~6% sodium hypochlorite (5.5% on average NaClO) and 0.5~0.8% sodium hydroxide (0.6% on average NaOH). Moreover, the cleaning solutions as other consumables were

evaluated in consideration of the functional evaluation of the cuvettes, probes, and tubing wash cleaning power. And then, the quantitative evaluation that may affect precision, cross-contamination rate (carry-over rate), correlation coefficient, and linearity analysis was performed (Figure 1).

4. Functional evaluation procedure and criteria

With the imported LX-CS was loaded, ADC value (Analog Digital Convert; calculated as the amount of change in absorbance) was measured through the cell blank check. And after the imported LX-CS was replaced by the self-manufactured OC-CS under the same conditions, ADC value was measured through the cell blank check. For the 55th cell, ADC mean value is calculated using ADC value measured during the time between dispensing of purified water with the 3rd nozzle and absorbing of purified water with the 4th nozzle (four-cycle), and ADC mean value is used as the reference value [9]. The evaluation criteria for 55 cuvettes and 3 probe wash and tubing cleaning were based on $-500 \sim +500$ ADC value range specified in the Service Part Manual (Eiken chemical, Japan). However, in this study, to reflect the clear cleaning power of semi-disposal acryl cuvettes, $\pm 200\%$ difference was accepted when ADC value was less than 10, and $\pm 100\%$ difference was accepted when ABS value was 10 or more. This was to evaluate by applying more strictly the

Table 1. Work standard for artificial-stool manufacturing

Raw material powder		NOC buffer	
Materials	Proportion (%)	Materials	Concentration
Bean flour	15	DW	10 L
Weak flour	10.5	HEPES	20 mM
Strong flour	22	NaCl	0.90%
Sucrose	22	CyDTA	1.0 mM
Hb	30.5	NaN_3	0.10%
Total	100	BSA	0.10%
		4N NaOH	Adjusted to pH 7.0

(A)

(B)

Figure 1. The appearance of evaluation products.

criteria [10].

Reference value=ADC mean value=Four-cycle accumulation of ADC values÷4 cycles --- Equation (1)

Cell blank value= Log_{10} (Reference value÷ADC average)×10,000 ----- Equation (2)

5. Quantitative evaluation procedures and criterion

1) Precision analysis

Precision was estimated using low (level 1) and high concentration (level 2) of OC quality control materials (Lot 8X010) for OC sensor PLEDIA. The acceptable range was 122 to 175 ng/mL (mean 149 ng/mL) for low concentration level 1 for quality control and 386 to 522 ng/mL (mean 454 ng/mL) for high concentration level 2. The quality control material was stored in refrigeration (2~8°C) before use.

Precision was evaluated for repeatability, between runs, and between days, intermediate data. Also, the results were determined by analyzing five runs per day with duplicates for each concentration daily for five days, and a total of 25 times was performed. Precision was evaluated according to the method specified in the Clinical and Laboratory Standards Institute (CLSI) EP05-A3 [11]. The acceptable coefficient of variation (%CV) was <5.0%.

2) Linearity analysis

The five levels of standard materials for each concentration were manufactured using the calibrator material and diluent for OC sensor PLEDIA. The standard material was evaluated for linearity at the highest concentration of 1000 ng/mL, the quantitative limitation of OC sensor PLEDIA. And then diluted with a buffer solution, so that the specimen-to-buffer ratio can be respectively 4:0, 3:1, 2:2, 1:3, 0:4 for 5 levels of concentration (0.0, 250.0, 500.0, 750.0, 1000.0 ng/mL) according to CLSI EP06-A2 [12]. After each treatment with the imported LX-CS and the self-manufactured OC-CS, the standard materials were measured in duplicate. The correlation coefficient and regression

line were calculated between the expected value on the X-axis and the measured value on the Y-axis. The criterion were % difference <±10% and % recovery <±10% [13].

3) Carry-over analysis

Once per week for 5 weeks, carry-over rate was determined by low (level 1) and high concentration (level 2) of OC quality control materials (Lot 8X010) for OC sensor PLEDIA replaced high-concentration and low-concentration of patient specimens. After the high concentration, OC control level 2 (H1, H2, H3, H4) were measured four times, and the low concentration OC control level 1 (L1, L2, L3, L4) were also measured four times in a row. Carry-over rate was calculated by $[L1-(L3+L4)/2]/[(H2+H3)/2-(L3+L4)] \times 100$ [14], and the result was considered acceptable if the rate was <±1.0%.

4) Correlation analysis

After preparing 7 normal samples and 33 abnormal samples of various concentrations, they were homogeneously pre-treated using a sampling bottle under the same conditions as the test method performed by the inspector during the collection process. And then they were measured after standing at room temperature. According to CLSI EP09-A3 [15], they were treated with the imported LX-CS and the self-manufactured OC-CS to analyze the correlation. A total of 40 artificial-stools of various concentrations were prepared in the analytical measurement range of 50 to 1000 ng/mL, and the results were estimated for 5 runs per day to obtain averages. In the case of more than 1000 ng/mL, it was considered as 1000 ng/mL, and a linear regression graph with the correlation coefficient(r) was calculated. And then, the *P*-values through t-test, the mean differences, the percent difference [% difference=Difference/(mean1+mean2)/2×100], and the difference: standard deviation ratio (D: ±1 SD ratio) were obtained.

Table 2. ADC value comparison of LX detergent cleaning solution and OC detergent cleaning solution through cell blank check for 10 days (unit: ADC value)

Cell No.	1 day			2 day			3 day			4 day			5 day			6 day		
	LX-CS (ADC)	OC-CS (ADC)	Diff (%)	LX-CS (ADC)	OC-CS (ADC)	Diff (%)	LX-CS (ADC)	OC-CS (ADC)	Diff (%)	LX-CS (ADC)	OC-CS (ADC)	Diff (%)	LX-CS (ADC)	OC-CS (ADC)	Diff (%)	LX-CS (ADC)	OC-CS (ADC)	Diff (%)
1	51	55	-4	48	47	1	51	50	1	48	52	-4	58	53	-5	62	62	0
2	71	62	9	62	67	-5	72	63	9	68	72	-4	64	64	0	74	74	0
3	51	49	2	49	50	-1	52	50	2	50	51	-1	50	48	-2	57	57	0
4	49	42	7	57	43	14	49	58	-9	44	51	-7	46	35	-11	41	38	3
5	32	30	2	30	33	-3	32	32	0	34	32	2	33	31	-2	41	40	1
6	35	33	2	33	35	-2	35	34	1	36	35	1	35	33	-2	46	45	1
7	71	71	0	71	81	-10	70	72	-2	72	72	0	73	69	-4	74	72	2
8	28	26	2	27	28	-1	29	29	0	29	29	0	30	28	-2	38	37	1
9	59	63	-4	56	53	3	59	57	2	53	60	-7	67	61	-6	66	65	1
10	92	103	-11	107	98	9	94	109	-15	99	93	6	113	109	-4	115	113	2
11	54	41	13	42	39	3	54	43	11	40	56	-16	42	45	3	52	50	2
12	28	23	5	31	23	8	28	32	-4	23	28	-5	27	19	-8	26	24	2
13	145	145	0	144	143	1	145	145	0	143	146	-3	145	144	-1	147	145	2
14	79	77	2	77	79	-2	79	78	1	80	80	0	81	79	-2	86	84	2
15	80	78	2	81	80	1	80	81	-1	80	80	0	80	79	-1	84	83	1
16	52	51	1	53	53	0	52	53	-1	53	53	0	53	52	-1	57	56	1
17	37	35	2	34	37	-3	37	37	0	38	38	0	38	35	-3	43	41	2
18	132	131	1	132	133	-1	133	134	-1	134	134	0	133	133	0	139	136	3
19	137	126	11	126	123	3	127	126	1	118	135	-17	122	132	10	133	130	3
20	26	26	0	26	27	-1	27	28	-1	28	28	0	28	24	-4	30	30	0
21	64	60	4	54	61	-7	65	56	9	58	67	-9	63	62	-1	65	67	-2
22	96	95	1	94	97	-3	98	96	2	98	98	0	97	93	-4	99	98	1
23	59	58	1	59	60	-1	60	60	0	60	60	0	61	58	-3	65	64	1
24	30	29	1	28	31	-3	31	30	1	32	31	1	32	28	-4	36	35	1
25	0	0	0	-2	0	-2	1	0	1	1	0	1	0	-3	-3	4	3	1
26	79	81	-2	82	87	-5	94	85	9	84	93	-9	90	78	-12	88	81	7
27	65	65	0	65	67	-2	66	66	0	67	67	0	67	65	-2	70	69	1
28	15	30	-15	16	25	-9	19	18	1	32	21	11	25	13	-12	26	18	8
29	30	31	-1	27	30	-3	31	29	2	33	31	2	29	27	-2	32	32	0
30	63	63	0	62	67	-5	70	65	5	66	70	-4	68	60	-8	70	67	3
31	66	64	2	63	70	-7	65	64	1	66	65	1	71	65	-6	73	69	4
32	22	20	2	19	22	-3	22	21	1	23	22	1	22	18	-4	26	25	1
33	14	13	1	11	15	-4	15	14	1	16	15	1	15	11	-4	18	18	0
34	111	97	14	93	91	2	83	96	-13	103	83	20	91	109	18	94	115	-21
35	105	107	-2	119	98	21	114	120	-6	111	114	-3	100	105	5	102	109	-7
36	9	3	6	200	-1	-2	6	2	4	200	9	-10	10	9	-1	11	12	-1
37	13	16	-3	-19	10	-4	14	13	1	17	15	2	14	10	-4	17	15	2
38	172	168	4	167	170	-3	173	173	0	175	175	0	174	173	-1	192	195	-3
39	88	86	2	86	90	-4	89	88	1	90	89	1	92	87	-5	96	94	2
40	27	27	0	27	28	-1	29	30	-1	30	29	1	30	25	-5	31	30	1

Table 2. Continued

Cell No.	1 day			2 day			3 day			4 day			5 day			6 day		
	LX-CS (ADC)	OC-CS (ADC)	Diff (%)	LX-CS (ADC)	OC-CS (ADC)	Diff (%)	LX-CS (ADC)	OC-CS (ADC)	Diff (%)	LX-CS (ADC)	OC-CS (ADC)	Diff (%)	LX-CS (ADC)	OC-CS (ADC)	Diff (%)	LX-CS (ADC)	OC-CS (ADC)	Diff (%)
41	67	66	1	2	65	70	-5	-7	69	68	1	1	69	64	-5	72	72	0
42	78	74	4	5	72	76	-4	-5	77	76	1	1	78	76	-2	79	84	-5
43	141	139	2	1	139	142	-3	-2	143	142	1	1	144	140	-4	124	142	-18
44	78	79	-1	-1	79	79	0	0	78	81	-3	-4	80	76	-4	77	83	-6
45	0	0	0	0	0	1	-1	-100	1	0	1	0	3	-2	-4	7	8	-1
46	59	53	6	11	50	52	-2	-4	48	53	-5	-9	56	57	5	54	64	-10
47	18	18	0	0	14	19	-5	-26	18	17	1	6	21	14	-5	24	24	0
48	117	108	9	8	103	115	-12	-10	123	106	17	16	113	116	-1	119	124	-5
49	22	21	1	5	19	23	-4	-17	23	22	1	5	24	19	-4	29	29	0
50	23	22	1	5	20	24	-4	-17	26	24	2	8	25	20	-5	29	31	-2
51	31	28	3	11	27	31	-4	-13	31	30	1	3	31	28	-3	35	38	-3
52	38	36	2	6	34	38	-4	-11	40	38	2	5	40	35	-5	44	44	0
53	21	20	1	5	17	21	-4	-19	22	21	1	5	23	18	-5	28	28	0
54	129	125	4	3	121	132	-11	-8	132	124	8	6	123	131	-2	137	139	-2
55	-1	0	-1	0	-2	0	-2	0	0	0	0	0	0	-2	-2	0	0	0

Cell No.	7 day			8 day			9 day			10 day			Mean (N=10)		
	LX-CS (ADC)	OC-CS (ADC)	Diff (%)	LX-CS (ADC)	OC-CS (ADC)	Diff (%)	LX-CS (ADC)	OC-CS (ADC)	Diff (%)	LX-CS (ADC)	OC-CS (ADC)	Diff (%)	LX-CS (ADC)	OC-CS (ADC)	Diff (%)
1	58	57	1	2	62	60	2	2	3	58	58	0	64	59	5
2	77	76	1	1	75	73	2	2	3	77	77	0	70	71	-1
3	58	57	1	2	58	58	0	0	0	58	58	0	55	56	-1
4	47	49	-2	-4	41	62	-21	-34	-34	47	48	-1	48	61	-13
5	44	42	2	5	41	42	-1	-2	-2	43	43	0	38	40	-2
6	48	46	2	4	47	47	0	0	0	48	48	0	44	45	-1
7	84	86	-2	-2	75	75	0	0	8	77	77	0	77	75	2
8	37	36	1	3	36	37	-1	-3	2	35	35	0	34	35	-1
9	58	59	-1	-2	67	63	4	6	4	59	55	4	70	64	6
10	113	112	1	1	115	121	-6	-5	5	114	109	5	117	121	-4
11	45	44	1	2	52	49	3	6	-1	46	46	0	46	48	-2
12	29	29	0	0	26	38	-12	-32	-32	30	31	-1	30	38	-8
13	144	147	-3	-2	149	147	2	1	1	148	148	0	149	148	1
14	86	86	0	0	87	86	1	1	1	87	88	-1	85	85	0
15	85	88	-3	-3	85	84	1	1	1	85	85	0	83	84	-1
16	58	60	-2	-3	58	58	0	0	0	59	59	0	56	57	-1
17	44	43	1	2	43	43	0	0	0	44	46	-2	42	42	0
18	136	137	-1	-1	139	135	4	3	3	137	138	-1	135	136	-1
19	125	123	2	2	133	127	6	5	5	125	124	1	126	131	-5

Table 2. Continued

Cell No.	7 day				8 day				9 day				10 day				Mean (N=10)			
	LX-CS (ADC)	OC-CS (ADC)	Diff	Diff (%)	LX-CS (ADC)	OC-CS (ADC)	Diff	Diff (%)	LX-CS (ADC)	OC-CS (ADC)	Diff	Diff (%)	LX-CS (ADC)	OC-CS (ADC)	Diff	Diff (%)	LX-CS (ADC)	OC-CS (ADC)	Diff	Diff (%)
20	32	34	-2	-6	32	33	-1	-3	32	32	0	0	30	32	-2	-6	29	29	0	-1
21	64	64	0	0	71	61	10	16	67	70	-3	-4	60	67	-7	-10	63	64	0	-1
22	100	100	0	0	101	100	1	1	102	102	0	0	101	100	1	1	99	98	1	1
23	66	66	0	0	66	66	0	0	66	66	0	0	64	65	-1	-2	63	62	0	0
24	37	36	1	3	37	37	0	0	37	37	0	0	35	36	-1	-3	34	33	1	2
25	4	3	1	33	4	4	0	0	5	5	0	0	2	3	-1	-33	2	2	0	27
26	90	84	6	7	95	87	8	9	83	96	-13	-14	86	81	5	6	87	85	2	2
27	71	70	1	1	71	70	1	1	71	71	0	0	69	70	-1	-1	68	68	0	0
28	27	36	-9	-25	23	21	2	10	20	26	-6	-23	20	19	1	5	22	23	0	-2
29	33	35	-2	-6	35	33	2	6	35	35	0	0	32	33	-1	-3	32	32	0	0
30	71	68	3	4	74	70	4	6	69	75	-6	-8	69	68	1	1	68	67	1	1
31	74	70	4	6	70	69	1	1	72	71	1	1	68	71	-3	-4	69	68	1	1
32	27	27	0	0	27	27	0	0	28	28	0	0	25	26	-1	-4	24	24	1	2
33	19	18	1	6	20	19	1	5	20	20	0	0	17	19	-2	-11	17	16	0	2
34	94	105	-11	-10	86	100	-14	-14	116	88	28	32	98	116	-18	-16	97	100	-3	-3
35	102	114	-12	-11	118	124	-6	-5	111	119	-8	-7	122	110	12	11	110	112	-2	-1
36	11	7	4	57	13	5	8	160	16	22	-6	-27	3	15	-12	-80	9	10	-1	-8
37	17	18	-1	-6	17	17	0	0	17	17	0	0	14	16	-2	-13	15	15	0	-1
38	193	195	-2	-1	190	190	0	0	193	191	2	1	183	187	-4	-2	181	182	-1	0
39	97	95	2	2	95	96	-1	-1	97	96	1	1	94	96	-2	-2	92	92	1	1
40	32	32	0	0	32	34	-2	-6	32	32	0	0	32	31	1	3	30	30	0	1
41	74	72	2	3	73	73	0	0	73	73	0	0	70	71	-1	-1	70	70	0	1
42	82	81	1	1	82	82	0	0	85	82	3	4	78	83	-5	-6	79	79	0	0
43	144	145	-1	-1	145	144	1	1	146	146	0	0	141	143	-2	-1	141	143	-2	-1
44	85	86	-1	-1	85	87	-2	-2	85	85	0	0	78	78	0	0	81	81	-1	-1
45	10	9	1	11	9	9	0	0	8	8	0	0	4	6	-2	-33	4	4	0	10
46	57	62	-5	-8	54	60	-6	-10	65	53	12	23	55	63	-8	-13	55	57	-2	-3
47	25	27	-2	-7	24	25	-1	-4	25	25	0	0	21	27	-6	-22	21	21	-1	-2
48	122	120	2	2	130	115	15	13	126	130	-4	-3	109	122	-13	-11	118	118	0	0
49	30	29	1	3	30	30	0	0	31	30	1	3	27	29	-2	-7	26	26	0	1
50	31	30	1	3	32	30	2	7	31	32	-1	-3	27	29	-2	-7	27	27	0	0
51	38	36	2	6	37	37	0	0	35	35	0	0	33	37	-4	-11	33	33	0	-1
52	44	43	1	2	45	44	1	2	44	44	0	0	41	44	-3	-7	41	41	0	1
53	28	27	1	4	28	28	0	0	27	26	1	4	25	27	-2	-7	24	24	0	2
54	137	131	6	5	127	132	-5	-4	131	125	6	5	128	130	-2	-2	130	129	1	1
55	0	0	0	0	0	0	0	0	0	0	0	0	-2	0	-2	0	-1	0	0	150

5) Statistical analysis

All statistical analyses were performed using MedCalc Software version 14.12.0 (Mariakerke, Belgium) and EP evaluator Release 11 (David G. Rhoads Assoc., Kennett Square, PA, USA).

RESULTS

1. Cuvettes cleaning check

OC Sensor PLEDIA was composed of 55 semi-disposal acryl cuvettes. The cleaning power of cuvettes was determined using the ADC value measured by optical absorbance. After conducting a sufficient cleaning solution prime under the same conditions for 10 days, a cell blank check was performed. After performing a sufficient cleaning solution prime under the same conditions once a day for 10 days, a cell blank check was evaluated. An analog digital conversion circuit was used to quantitatively measure the cleaning power of both products. If the ADC value was less than 10, it was allowed as a percentage difference of $\pm < 200$, and if the ADC value was more than 10, it was allowed as a percentage difference of $\pm < 100$. As a result of the measurement, all of 55 cuvettes satisfied the acceptance criteria (Table 2).

2. Precision

I calculated the mean, standard deviation, coefficient of variation for repeatability, between runs, between days, and intermediate data using the OC quality control materials. And as a result of evaluation by OC Sensor PLEDIA in LX-CS, low concentration material was 142.8 ± 5.3 ng/mL, 1.63%, 1.70%, 3.30%, 3.70% and

high concentration material was 442.1 ± 17.4 ng/mL, 1.01%, 1.00%, 3.80%, 3.90%. And in OC-CS, low concentration material was 145.1 ± 4.10 ng/mL, 1.90%, 1.90%, 2.10%, 2.80% and high concentration material was 445.4 ± 11.20 ng/mL, 1.23%, 1.20%, 2.20%, 2.50%, respectively (Table 3).

3. Linearity

As a result of using the OC calibrator, the imported LX-CS and the self-manufactured OC-CS showed excellent linearity within each analytical measurable range in the linear regression plots shown in Figure 2. The linearity regression of LX-CS was $y = 1.0017x - 5.7$ ($r^2 = 0.9982$) and the self-manufactured OC-CS was $y = 0.9924x - 2.7$ ($r^2 = 0.9996$), respectively.

4. Carry-over

I measured the high concentration OC control level 2 (H1, H2, H3, H4) four times and then the low concentration OC control level 1 (L1, L2, L3, L4) four times in a row. As a result of the imported LX-CS and the self-manufactured OC-CS were evaluated once a week for 5 days with OC sensor PLEDIA, the carry-over rates (%) of all results were within 1%, which is acceptable (Table 4).

5. Correlation

Based on the AMR of 50 to 1000 ng/mL, the total of 40 artificial-stool specimens of 7 normal samples and 33 abnormal samples were treated using OC detergent cleaning solution for 5 days and measured with OC Sensor PLEDIA (Table 5). Under the same conditions, they were also treated using LX detergent cleaning

Table 3. Analytical imprecision of LX detergent cleaning solution and OC detergent cleaning solution using internal quality control materials

Reagent	Level	Mean	SD	%CV			
				Repeatability	Between run	Between day	Intermediate
LX detergent cleaning solution	Level 1	142.8	5.30	1.63	1.70	3.30	3.70
	Level 2	442.1	17.40	1.01	1.00	3.80	3.90
OC detergent cleaning solution	Level 1	145.1	4.10	1.90	1.90	2.10	2.80
	Level 2	445.4	11.20	1.23	1.20	2.20	2.50

%CVs were calculated by EP evaluator Release 11.

Figure 2. Linearity analysis of LX detergent cleaning solution (A) and OC detergent cleaning solution (B).

Table 4. Carry-over for 5 days once a day of OC sensor PLEDIA

NO.	LX detergent cleaning solution									OC detergent cleaning solution								
	OC control level 2 (High)				OC control level 1 (Low)				Result	OC control level 2 (High)				OC control level 1 (Low)				Result
	H1	H2	H3	H4	L1	L2	L3	L4		H1	H2	H3	H4	L1	L2	L3	L4	
1	437	449	445	443	143	148	144	145	0.00%	458	460	457	457	151	150	150	154	0.33%
2	410	416	411	411	141	141	148	136	0.37%	435	438	438	433	143	143	146	142	0.34%
3	449	456	452	460	146	146	150	147	0.82%	443	449	447	444	145	145	144	146	0.00%
4	442	452	450	443	145	147	141	145	0.65%	436	438	449	449	145	145	145	141	0.67%
5	454	462	466	461	148	150	149	151	0.64%	460	476	470	459	156	155	158	154	0.00%

Table 5. Artificial-stool sample number for interval range and ratio

Artificial-stool	Total N=40 (50~1000 ng/mL)					
	0~100	101~200	201~500	501~700	701~900	>901
	7 (17.5%)	9 (22.5%)	5 (12.5%)	14 (35%)	3 (7.5%)	2 (5%)

solution. The result of linear regression graph was $y=0.9978x-6.5683$, where $r^2=0.9997$. Besides, when the averages of 40 artificial-stool specimens were compared, the % difference was less than 10, and the P -value was less than 0.1, showed little difference between the two products. The ratio of ± 1 SD based on % difference showed similar results for both products (Table 6).

DISCUSSION

For colorectal cancer screening, many general medical institutions use the OC Sensor series as a

quantitative method [16]. Depending on the OC sensor series, OC Sensor PLEDIA requires LX detergent cleaning solution, buffer solution, and OC Diana latex reagent. Especially, LX detergent cleaning solutions are responsible for cleaning cuvettes, nozzles, and probes cleaning. As for the functional analysis of the imported LX detergent cleaning solution (LX-CS) and the self-manufactured OC detergent cleaning solution (OC-CS), the role of cleaning in each instrument part plays a very important role in results stability. To check the cleaning power, there were cases where the contamination rate was measured by evaluating the residual amount per unit area or calculating the ppm,

Table 6. Mean, % difference, *P*-value, and % difference: ± 1 SD ratio using artificial-stool

No.	Mean (ng/mL)		Diff (ng/mL)	Diff (%)	<i>P</i> -value by t-test	Diff (%): ± 1 SD ratio	
	LX-CS	OC-CS				LX-CS	OC-CS
1	97	90	7.0	7.8	<i>P</i> <0.0001	1.1	1.1
2	97	93	4.0	4.3	<i>P</i> =0.0001	1.3	1.1
3	85	80	5.0	6.3	<i>P</i> =0.0001	1.1	1.6
4	100	96	4.0	4.2	<i>P</i> =0.0384	0.5	0.8
5	87	83	4.0	4.8	<i>P</i> =0.0021	1.2	1.0
6	91	84	7.0	8.3	<i>P</i> <0.0001	3.0	2.9
7	111	106	5.0	4.7	<i>P</i> =0.0003	1.1	1.2
8	86	82	4.0	4.9	<i>P</i> =0.0003	1.8	2.1
9	103	102	1.0	1.0	<i>P</i> =0.0698	0.2	0.1
10	141	134	7.0	5.2	<i>P</i> <0.0001	0.9	1.2
11	140	133	7.0	5.3	<i>P</i> =0.0001	1.1	1.1
12	154	147	7.0	4.8	<i>P</i> <0.0001	1.1	1.2
13	150	143	7.0	4.9	<i>P</i> <0.0001	0.9	1.1
14	144	135	9.0	6.7	<i>P</i> <0.0001	2.0	1.5
15	143	135	8.0	5.9	<i>P</i> <0.0001	1.3	1.7
16	214	209	5.0	2.4	<i>P</i> =0.0059	0.5	0.6
17	207	199	8.0	4.0	<i>P</i> =0.0007	0.8	1.1
18	225	212	13.0	6.1	<i>P</i> =0.0002	0.7	0.9
19	212	207	5.0	2.4	<i>P</i> =0.0203	0.3	0.5
20	146	140	6.0	4.3	<i>P</i> =0.0047	0.8	1.0
21	146	143	3.0	2.1	<i>P</i> =0.0484	0.3	0.5
22	340	323	17.0	5.3	<i>P</i> =0.0032	0.6	0.7
23	539	527	12.0	2.3	<i>P</i> =0.0157	0.6	0.6
24	512	508	4.0	0.8	<i>P</i> =0.0636	0.1	0.2
25	550	543	7.0	1.3	<i>P</i> =0.0402	0.4	0.4
26	562	552	10.0	1.8	<i>P</i> =0.0236	0.6	0.5
27	606	595	11.0	1.8	<i>P</i> =0.0290	0.5	0.6
28	619	614	5.0	0.8	<i>P</i> =0.0680	0.2	0.3
29	595	580	15.0	2.6	<i>P</i> =0.0062	0.7	0.7
30	595	585	10.0	1.7	<i>P</i> =0.0803	0.3	0.5
31	579	570	9.0	1.6	<i>P</i> =0.0258	0.4	0.5
32	611	606	5.0	0.8	<i>P</i> =0.0879	0.2	0.2
33	642	631	11.0	1.7	<i>P</i> =0.0035	0.5	0.5
34	660	641	19.0	3.0	<i>P</i> =0.0002	0.8	0.8
35	771	755	16.0	2.1	<i>P</i> =0.0245	0.5	0.5
36	824	820	4.0	0.5	<i>P</i> =0.0940	0.1	0.1
37	889	880	9.0	1.0	<i>P</i> =0.0076	0.1	0.2
38	522	512	10.0	2.0	<i>P</i> =0.0266	0.3	0.3
39	986	991	-5.0	-0.5	<i>P</i> =0.0230	-0.2	-0.4
40	997	995	2.0	0.2	<i>P</i> =0.0885	0.3	0.2

P-values were calculated by MedCalc Software version 14.12.0.

but in this paper, it was replaced with the ADC value [17, 18]. And then the quantitative cleaning power of each instrument part can be determined by measuring the ADC value based on the Technical Service Manual (Eiken chemical, Japan). The percent differences were compared to the ADC values of 55 cuvettes concerning the cell blank value for 10 days, to see if there was any difference in functional analysis of the cleaning power

of LX-CS and OC-CS. The ADC values of 10 or less showed a rather high % difference, but this seems to be a relatively large bias difference for low values. Comparing the functional analysis differences between OC-CS and LX-CS, they were all acceptable given the acceptance range of ADC 10 or less ± 200 % difference and ADC 10 or more ± 100 % difference (Table 2). Besides, before this, the equivalence was confirmed

using component analysis through LC-MS.

The %CVs of repeatability and within-laboratory precision using low concentration and high concentration were performed. When the coefficients of variation that were tested for repeatability, between runs, between days, and in intermediate data were compared, LX-CS and OC-CS were both less than 5.0% in high and low concentrations of quality control materials, showed excellent results. As a result of evaluating OC Sensor PLEDIA using LX-CS, the intermediate coefficient of variation was 3.50% for low concentration materials, and 3.61% for high concentration materials, showed good precision results in low concentration. As a result of evaluating OC Sensor PLEDIA using OC-CS, the intermediate coefficient of variation was 2.70% for the low-concentration materials and 2.33% for high-concentration materials, which showed better precision in high-concentration materials. Regarding precision, OC-CS showed a little higher precision than the LX-CS, and both products were less than 5.0%, which was excellent quality for quantitative validation performance. In a previous study that evaluated OC NEO and OC DIANA among the OC sensor series, it can be seen that the precision was higher than that measured at 7.8% and 3.1% for low-concentration materials, and 3.5% and 2.0% for high-concentration materials, respectively [19]. Besides, in a previous study evaluating HM-JACK (Kyowa Medex, Japan) and OC IO (Eiken Chemical, Japan) at various concentrations, repeatability of positive specimens using HM JACK is 3.5% in high concentration materials and 4.8% in low concentration specimens [20]. And then repeatability using OC sensor IO was 2.0~4.3% in various levels of concentration [21]. As shown in Table 3, the precision performance was excellent in this study.

Linearity analysis of LX-CS and OC-CS measured with OC sensor PLEDIA had r^2 values of 0.9982 and 0.9996, respectively, which showed excellent linearity in all concentration ranges. However, both products showed a slightly higher % difference, 3.0~6.0% in the

middle range of AMR 50 to 1000 ng/mL. These results were similar to those in previous studies in which OC sensor series were evaluated, and it was thought that there was no significant difference in linearity between LX-CS and OC-CS [22].

Since correlation between LX-CS and OC-CS cannot be handled with clinical samples, the results of using artificial-stool samples for external quality control materials, coefficients of determination between two products were excellent at $r^2=0.9997$. In a linear regression analysis, LX-CS comparing with OC-CS was shifted upward to 6.568 ng/mL, confirmed that it was lower than OC-CS in the same sample, which was consistent with the linearity evaluation compared to correlation (Figure 3). These results could not be seen as a significant difference when the site variation of the collection process was reflected in consideration of the characteristics of inhomogeneous raw materials, small sample volume, calibration curve change according to the different lot, and could be confirmed through similar studies [23]. When LX-CS and OC-CS were compared, the proportion of artificial specimens of 500~700 ng/mL was the highest (Table 5). In correlation evaluation, t-test showed no significant difference ($P<0.1$). As the results of mean and ratio of

Figure 3. The correlation of hemoglobin (Hb) concentrations quantified by LX detergent cleaning solution and OC detergent cleaning solution.

% difference versus $\pm 1SD$ were calculated for five days, there was no difference in an acceptable range for the two products. Therefore, the results showed no significant difference in the quantitative analysis between the two products. Also, the equation used based on the acceptance criteria for the two products using the equation as shown in Table 6 could be used the % difference normally cited in Korea or the range validation formula described in CLSI C54-A. However, since the range validation formula was similar to % difference except for determining the number repeatability, the % difference was applied in this study [24].

In this study, the imported LX detergent cleaning solution and the self-manufactured OC detergent cleaning solution showed excellent precision and linearity in fecal occult blood test using OC Sensor PLEDIA, and both products showed excellent quantitative performance due to the low carry-over rate using commercial materials. Also, in the functional analysis of two products, cleaning power and correlation using artificial-stools showed a high equivalency between two products. I suggested that showed no significant difference between LX-CS and OC-CS based on quantitative data.

In conclusion, this study clearly showed that the self-manufactured OC detergent cleaning solution than the imported LX detergent cleaning solution is a good choice since it could provide a faster and more stable supply to domestic OC Sensor PLEDIA users than the imported LX detergent solution. However, the evaluation period was relatively short when considering the functional analysis, and it was recognized that additional research using patient samples was necessary.

요 약

이 연구의 목적은 기능적, 정량적 분석을 바탕으로 수입 LX 세정액(LX-CS)과 자가제조 OC 세정액(OC-CS)의 성능을 비교하였다. 기능적 분석은 ADC값을 사용하여 평가하였다. 정량

적 분석을 위해 CLSI 지침에 따라 정도 관리 물질을 이용하여 정밀도, 직선성 및 오염률을 평가하였다. OC-Sensor PLEDIA (Eiken Chemical, Japan)를 이용한 모든 cuvette의 ADC값은 허용기준을 만족하였다. 정량적 분석의 경우 두 제품의 정밀도는 5.0% 미만이었으며, 오염률은 $\pm 1.00\%$ 이하였다. 직선성 기울기와 r^2 값은 LX-CS에서 각각 1.0017와 0.9982였으며, OC-CS에서 0.9924와 0.9996이었다. 상관계수(r)는 0.9997이었다. 또한 40개의 가변을 이용한 % difference는 10% 미만, P 값은 0.1 미만이었다. 각각 차이: 표준 편차 비($D: \pm 1 SD$ 비)에 기초한 두 제품 모두 유사한 결과를 보였다. 결론적으로 두 제품의 기능적, 정량적 분석을 비교한 결과 유사하였다. 향후 자가 제조한 OC세정액은 수입 LX세정액보다 훨씬 더 안정적이고 빠른 공급을 제공할 수 있을 것이라 기대된다.

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