Original Article

Assessment of possible disruptive effects of residues from cleaning agents on medical devices after cleaning on protein assays

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Abstract

The most important analyte used to verify the cleanliness of a medical device after a cleaning process is protein [1, 2]. There are a variety of different Assays to test for protein, the most commonly used Assays are based on ortho-phthalaldehyde (OPA) and bicinchoninic acid (BCA) [2]. In this study 42 different cleaning detergents from 14 different manufacturers were tested for their potential to influence the output of these Assays. Serial dilutions of these detergents ranging from 1 % to 0.001 % were tested. The matrix 1 % alkalized (pH 11.0) sodium dodecyl sulfate (SDS) solution was chosen, as this is one of the suggested and established extraction fluids for medical devices [3].

Five of these tested detergents showed a high potential risk of affecting the performance of the OPA Assay. The results were over 100 µg/ml expressed as bovine serum albumin (BSA) equivalent at a concentration of 0.031 %. 17 detergents showed a medium potential risk with results higher than 10 μg/ml at a concentration of 0.25 %, thus only influencing the signal at a comparatively high concentration. 20 detergents were not significantly affecting the OPA Assay (results < 10 μg/ml at a concentration of 0.25 %).

For the BCA Assay, only one detergent showed a high potential risk with a result of over 100 µg/ml at a concentration of 0.031%. Five detergents showed a medium potential risk with results higher than 10 μg/ml at a concentration of 0.25%. 36 detergents were not significantly affecting the BCA Assay (results < 10 μg/ml at a concentration of 0.25%).

Overall, the OPA Assay was more susceptible to interferences from cleaning detergents than the BCA Assay. With the approach described it is possible to identify the risk if an Assay might be impaired by residuals of the cleaning detergent after a cleaning process.

Keywords
- Protein Assay
- Cleaning detergent
- Medical device

Introduction

The most important analyte used to verify the cleanliness of a medical devi ce after a cleaning process is protein [1, 2]. There are a variety of different Assays to test for protein, the most commonly used Assays are based on ortho-phthalaldehyde (OPA) and bicinchoninic acid (BCA) [2]. A cleaning process, be it manual or automated, for a medical device usually incorporates the use of cleaning detergent. Cleaning detergents contain a variety of ingredients to facilitate cleaning, e.g. enzymes, surfactants, complexing agents, buffers and so on. Naturally, depending on how much of these chemicals remain on a medical device after a cleaning process, they can interfere with the Assays used to verify cleanliness and influence the interpretation of the results. One approach to investigate these disruptive effects is the use of negative device controls during a cleaning validation [4]. Nonetheless, the general potential risk that residual cleaning detergents on medical devices after cleaning impact the Assays can be investigated by testing these cleaning detergents directly. For this purpose serial dilutions of 42 different detergents ranging from 1% to 0.001 % were tested. The matrix

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1% alkalized (pH 11.0) sodium dodecyl sulfate (SDS) solution was chosen, as this is one of the suggested and established extraction fluids for medical devices [3].

Production of the Serial Dilutions of the Detergents

A stock solution (1 %m) of 99 g 1% alkalized (pH 11.0) sodium dodecyl sulfate (SDS) solution and 1 g detergent (see Table 2) was prepared in a 100 ml glass beaker on a scale. This solution was stirred with a stir bar on a magnetic stirrer for five minutes. Afterwards this stock solution was serially diluted down to a concentration of 0.001% with 1% alkalized (pH 11.0) SDS solution (see Fig. 1 and Table 1). Each solution was mixed for 10 s on a vortex before the next dilution step.

An aliquot of each prepared concentration was evaluated with the Assays described below.

Protein Assays

OPA Assay [5]

The working reagent (1-fold concentrated) for the OPA Assay was produced based on Wehrl, Kircheis [6] with the adjustment of using 116 mg Sodium 2-mercaptoethanesulfonate as thiol component per 50 ml of working reagent.

The modified OPA Assay (OPA= ortho-phthaldialdehyde) is a quantitative method to determine the concentration of proteins with **free α- and ε-terminal amino (-NH_2) groups** in a solution. The OPA Assay is based on the chemical reaction of free amino groups with ortho-phthaldialdehyde in the presence of a thiol compound to fluorescent isoindoles, which can be detected spectrophotometrically at 340 nm (absorbance maximum). Alternatively, the emission of the fluorescent isoindoles can be detected at 455 nm.

For each test series, calibration (linear regression) was performed with BSA (bovine serum albumin) standard solutions which are prepared in 1% SDS solution ranging from 0 µg/ml to 50 µg/ml. The standards were mixed in a 1:1 part ratio with the working reagent, incubated for 3 min at 37 °C and the absorbance was read at a wavelength of 340 nm. Each measurement was performed in triplicate. The absorbance was corrected for its corresponding blank.

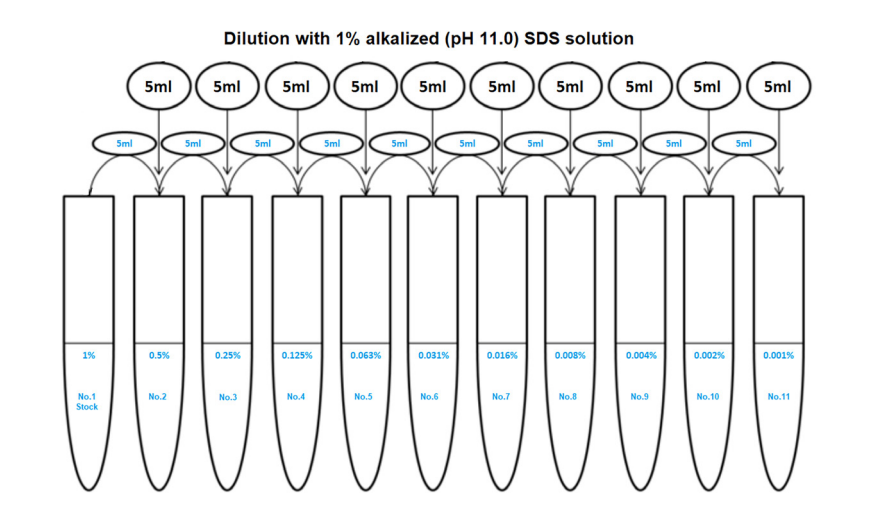

Figure 1: Scheme of the serial dilution

Table 1: Scheme of the serial dilution

Solution No.	c [%]	Volume solution	Volume 1 % SDS solution
1 (Stock)	1.000	5 ml of stock solution No.1	-
2	0.500	5 ml of solution No. 1	5 ml
3	0.250	5 ml of solution No. 2	5 ml
4	0.125	5 ml of solution No. 3	5 ml
5	0.063	5 ml of solution No. 4	5 ml
6	0.031	5 ml of solution No. 5	5 ml
7	0.016	5 ml of solution No. 6	5 ml
8	0.008	5 ml of solution No. 7	5 ml
9	0.004	5 ml of solution No. 8	5 ml
10	0.002	5 ml of solution No. 9	5 ml
11	0.001	5 ml of solution No. 10	5 ml

linear fit (y=a + b*x) – OPA Assay

Table 3: Specifications of the exemplary calibration for the OPA Assay

Parameter	Value
N	10
m	3
intercept (a) [A]	0.0036
slope (b) [A]/[µg/ml]	0.0029
factor 1/b	350.1
lin. correlation coefficient R	0.99993
coefficient of determination R^2	0.99985
critical value yk [A]	0.0056
limit of detection [µg/ml]	0.9
LOQ limit of quantification (error <= 33.33%, k=3) [µg/ml]	3.2

Table 2: Tested cleaning detergents

Detergent	Manufacturer	ID	REF	LOT
Cleaner N	BBraun	CN	3893146	17112M05
Helimatic Cleaner alkaline	BBraun	HCA	18731	17291M02
Helimatic Cleaner neutral	BBraun	HCN	18519	17071M07
Helizyme	BBraun	HZ	18767	17064M14
Stabimed	BBraun	SM	18779	13474M13
Stabimed fresh	BBraun	SMF	19690	17192M07
deconex Twin Zyme	Borer Chemie AG	TZ	510605.00-K10W	387724
deconex Twin PH10	Borer Chemie AG	TP	531910.00-K10W	386175
deconex Prozyme Alka-x	Borer Chemie AG	PAX	525000.00-KK5W	398607
deconex Prozyme active	Borer Chemie AG	PA	524705.00KK5W	398606
deconex Power Zyme	Borer Chemie AG	PZ	REF.96296	340243
deconex 28 Alka One-x	Borer Chemie AG	AOX	518215.00-K10W	369904
Decon90	Decon Lab	DN	DCND901	170118.A
ASP Cidezyme	Johnson & Johnson	AZ	2258	82053A-0111
neodisher MediClean	Dr. Weigert	MC	WE404333	633069/0419
neodisher MediClean Dental	Dr. Weigert	MCD	WE405333	576278/0617
neodisher FA	Dr. Weigert	FA	WE410133	586565/1117
neodisher MediClean Forte	Dr. Weigert	MCF	WE405033	630440/0419
neodisher MediZym	Dr. Weigert	MZ	WE404033	540485/0216
neodisher SeptoClean	Dr. Weigert	SC	V402533-0916-WE	609597/0518
neodisher PreStop	Dr. Weigert	PS	V408659-0418-WE	620942/1118
neodisher Z Dental	Dr. Weigert	ZD	WE405533	565876/0217
neodisher Septo DN	Dr. Weigert	SDN	V401033-0716-WE	622468/1118
neodisher N	Dr. Weigert	NN	WE420133	529639/0815
neodisher Z	Dr. Weigert	NZ	WE420233	547589/0516
Olympus EndoAct	Ecolab	EA	3056690WD00212A	3338AP0613
Olympus EndoDent Plus	Ecolab	EDP	3077150WD00230A	2358AP1007
Getinge Clean Rinse Aid	Getinge	RA	503435405	1747-93533
Getinge Clean Enzymatic Detergent	Getinge	ED	503437005	1769-96272
Getinge Clean Universal Detergent	Getinge	DU	6001692401	1777-10095
Getinge Clean Manual Plus	Getinge	MP	6001821001	B607098
RY-0501	Miura	RY	RY-0501	3258F
Gigazyme	Schülke & Mayr	GZ	2527-A	1503119
Thermosept EndoCleaner	Schülke & Mayr	TEC	20001076-A	1502754
Thermosept PPA additive	Schülke & Mayr	PPA	2000219-B	1514844
Thermosept PPA base	Schülke & Mayr	PPB	125702	1503401
Thermosept x-tra	Schülke & Mayr	TX	127605	1521930
Steelco Zyme	Steelco	SZ	-	17292
Prolystica Enymatic 2x	Steris	EX	1C33-T4PE	28000
Prolystica Detergent Neutral UC	Steris	DNU	1C07-T6WR	286138
Prolystica Neutral Detergent 2x	Steris	NDX	1C032-T4PE	295584
Prolystica Enzymatic Cleaner UC	Steris	ECU	1C16-T4	284486

An exemplary set of calibration data for one test series is given in Table 3 and Fig. 2. Measurements and data acquisition were performed with a spectrophotometer (Type Analyzer Gallery) from ThermoFisher. The evaluation of the data was performed with the integrated software of the Analyzer as well as QSM according to DIN 32645 [7].

BCA Assay [8]

For the Biuret/BCA Assay a commercially available test kit was utilized (BC Assay Protein Quantitation Kit, Uptima /Interchim, REF UP40840A).

The Biuret/BCA Assay is a colorimetric method: it involves the reduction of Cu^{2+} to Cu^{+} by the **peptide bonds** of proteins in an alkaline medium. The bicinchoninic acid (BCA) chelates Cu^{+} ions with very high specificity to form a water-soluble purple-colored complex. The reaction is read at a defined time and temperature condition as the purple-colored copper BCA complex will continually develop. However, the procedural development is slow enough to allow the processing of numerous samples. The absorbance of the colored complex can be detected spectrophotometrically in a range of 540 nm to 590 nm with a maximum at 562 nm.

For each test series, calibration (quadratic regression) was performed with BSA (bovine serum albumin) standard solutions which were prepared in 1% SDS solution ranging from 0 µg/ml to 50 µg/ml. The standards were mixed in a 1:1 part ratio with the working reagent, incubated for 60 min at 37 °C and the absorbance was read at a wavelength of 675 nm. The absorbance was corrected for its corresponding blank. Each measurement was performed in triplicate.

An exemplary set of calibration data for one test series is given in Table 4 and Fig. 3. Measurements and data acquisition were performed with a spectrophotometer (Type Analyzer Gallery) from ThermoFisher. The evaluation of the data was performed with the integrated software of the Analyzer as well as QSM according to DIN 32645 [7].

Results

Three arbitrary categories (High, Medium and Low) to classify the output of the different detergents were set. The detergents with a "High" output (see Table 5 and 6) deliver a result equivalent to over 100 µg/ml at a concentration of

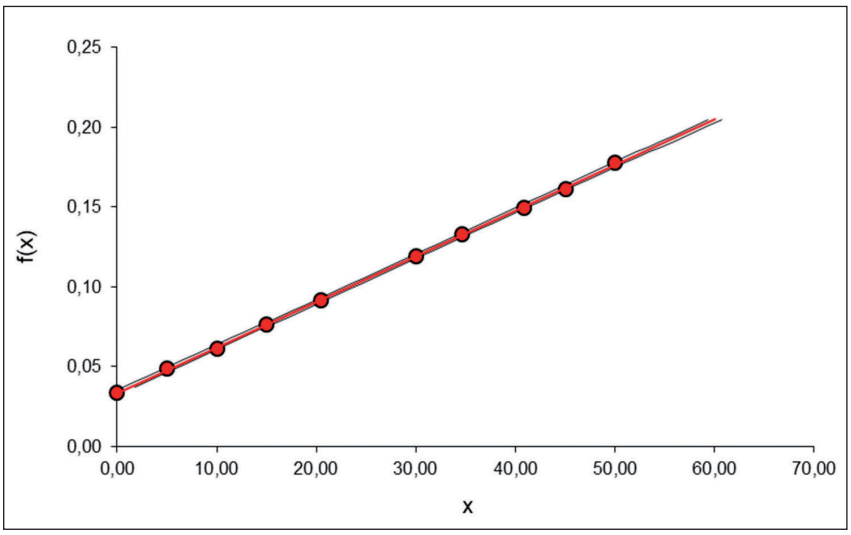

Figure 2: Exemplary calibration curve for the OPA Assay with confidence interval

quadratic fit (y=a + b*x + c*x²) - BCA Assay

Table 4: Specifications of the exemplary calibration for the BCA Assay

Parameter	Value
N	10
m	3
a [A]	0.070787
b [A]/[µg/ml]	0.014770
c [A]/[µg/ml]²	-0.000053
factor 1/b	67.7
critical value yk [A]	0.0070
limit of detection [µg/ml]	1.4
LOQ limit of quantification (error <= 33.33%, k=3) [µg/ml]	4.6

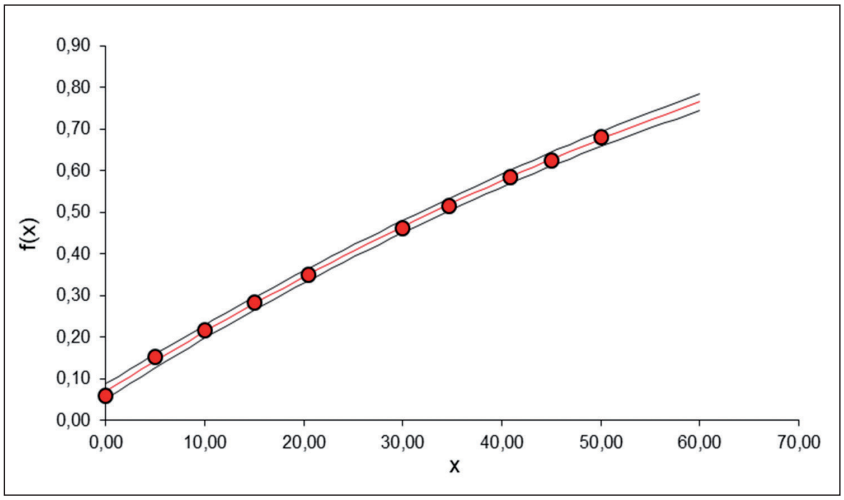

Figure 3: Exemplary calibration curve for the BCA Assay with confidence interval

Detergents with a "High" output (over 100 µg / ml at 0.031 %)
OPA Assay ➔ SM, SMF, EX, TX, TEC

Table 5: Detergents with a "High" output (OPA Assay)

No.	c [%]	SM	SMF	EX	TX	TEC
1	1.000	>max*	>max*	>max*	>max*	>max*
2	0.500	>max*	>max*	>max*	>max*	>max*
3	0.250	>max*	>max*	>max*	1039.9	1027.3
4	0.125	995.2	820.5	864.4	543.7	539.1
5	0.063	518.5	424.2	433.3	274.1	263.1
6	0.031	260.9	204.8	209.2	128.9	129.6
7	0.016	131.0	103.5	100.8	61.6	60.6
8	0.008	63.2	50.6	50.3	30.2	31.5
9	0.004	31.3	25.0	22.6	14.4	15.1
10	0.002	15.4	12.6	10.5	7.0	7.8
11	0.001	8.0	7.0	4.5	3.6	4.1

BCA Assay ➔ PPB

Table 6: Detergents with a "High" output (BCA Assay)

No.	c [%]	PPB
1	1.000	>max*
2	0.500	>max*
3	0.250	>max*
4	0.125	>max*
5	0.063	>max*
6	0.031	>max*
7	0.016	241.6
8	0.008	126.1
9	0.004	62.1
10	0.002	27.4
11	0.001	13.2

* ">max" means that the signal exceeds the upper capacity of the detection system

Detergents with a "Medium" output (over 10 µg / ml at 0.25 %)
OPA Assay -> DNU, AOX, ECU, NDX, MCD, DU, MP, TZ, MC, PZ, SZ, AZ, HZ, MCF, RY, PA, PAX

Table 7: Detergents with a "Medium" output (OPA Assay)

No.	c [%]	DNU	AOX	ECU	NDX	MCD
1	1.000	832.4	217.3	156.9	146.7	136.9
2	0.500	415.5	106.3	112.7	71.5	66.1
3	0.250	205.8	51.1	59.0	35.3	33.3
4	0.125	99.9	24.6	31.6	16.8	16.9
5	0.063	49.1	12.2	14.0	8.2	8.2
6	0.031	22.6	5.9	6.3	3.5	4.4
7	0.016	10.5	<LOQ	<LOQ	<LOQ	<LOQ
8	0.008	4.7	<LOQ	<LOQ	<LOQ	<LOQ
9	0.004	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
10	0.002	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
11	0.001	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

0.031 %. This category has a high risk to distort the assessment of cleaned devices if the respective Assay is used and if the detergent is not rinsed off properly and even low amounts of detergent remain on the device. For the OPA Assay five of the detergents tested fall into this category and for the BCA Assay one detergent.

Detergents with a "Medium" output (see Table 7 and 8) deliver a result equivalent to over 10 μg/ml at a concentration of 0.25%. Since the detergents in this category only show an elevated signal at comparably high concentrations, it is not very likely that the Assays used to assess the cleanliness of devices, if rinsed properly, are influenced due to residual detergent. For the OPA Assay seventeen of the detergents tested fall into this category and five detergents for the BCA Assay. For the OPA Assay only the five result tables of the detergents with the highest output in this category are shown.

Detergents with a "Low" output (see Table 9 and 10) deliver a result equiv-

alent to below 10 µg/ml at a concentration of 0.25%. Most of the tested detergents fall into this category: twenty for the OPA Assay and thirty-six for the BCA Assay. Since the detergents in this category do not show a significant signal even at a higher concentration with the respective assay it is unlikely that residual detergent on a cleaned device creates a false-positive result. Only the five result tables of the detergents with the highest output in this category are shown for each assay.

Discussion

The data provided in this study is not meant to judge the performance of cleaning detergents, whether it be their cleaning capability or their rinse ability. It is meant to make an educated assessment if there is a possible risk that the cleaning detergent is influencing the verification of cleanliness of a medical device after a cleaning process or if the Assays used for this verification might be impaired. This is valuable information for manufacturers of medical devices who have to validate their cleaning instructions, for testing laboratories and even for technicians in the field who have to do cleaning performance qualification testing. In general, if a medical device is properly rinsable and properly rinsed, no increased amounts of residual cleaning detergent are to be expected on the medical device. Additionally, most detergents only show a negligible amount of interference on the Assays tested. In general, the OPA Assay was more susceptible to interferences from cleaning detergents than the BCA Assay. It is important to know about these possible interferences to avoid misinterpretations of results from in theory adequately cleaned medical devices. The data in this study only applies to the Assays exactly as described and only to the matrix 1 % SDS solution. Different protein Assays, e.g. based on Coomassie Brilliant Blue, or even the same Assay but a different test kit will probably show a different output. Assays for completely different markers will also very likely react differently to the tested detergents. A different extraction fluid might show a different output as well, if the observed data in this study is not an interference between the detergent and the chemicals from the Assays directly rather than an incompat-

BCA Assay ➔ ECU, SMF, TZ, MP, EX

Table 8: Detergents with a "Medium" output (BCA Assay)

No.	c [%]	ECU	SMF	TZ	MP	EX
1	1.000	131.7	63.1	63.1	51.2	32.5
2	0.500	56.4	27.8	28.7	23.9	18.0
3	0.250	25.8	16.8	15.2	12.8	10.3
4	0.125	14.9	8.9	8.0	7.0	5.9
5	0.063	8.2	5.0	<LOQ	<LOQ	<LOQ
6	0.031	4.6	<LOQ	<LOQ	<LOQ	<LOQ
7	0.016	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
8	0.008	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
9	0.004	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
10	0.002	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
11	0.001	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

Detergents with a "Low" output (below 10 µg / ml at 0.25 %)
OPA Assay ➔ PS, ED, HCA, MZ, CN, GZ, EDP, DN, TP, HCN, FA, SC, ZD, SDN, PPA, NN, NZ, EA, RA, PPB

Table 9: Detergents with a "Low" output (OPA Assay)

No.	c [%]	PS	ED	HCA	MZ	CN
1	1.000	32.1	18.4	11.0	11.7	9.5
2	0.500	15.8	9.3	5.6	5.5	4.8
3	0.250	7.7	4.7	3.4	<LOQ	<LOQ
4	0.125	3.7	<LOQ	<LOQ	<LOQ	<LOQ
5	0.063	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
6	0.031	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
7	0.016	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
8	0.008	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
9	0.004	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
10	0.002	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
11	0.001	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ

ibility with the 1 % SDS solution. With the approach described it is possible to identify the risk if an Assay might be impaired by residuals of the cleaning detergent after a cleaning process. In the case of impairment, a different Assay to verify cleanliness can be used.

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The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products.

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BCA Assay ➔ AZ, SDN, PZ, MZ, RY, SZ, PAX, PA, HZ, RA, TX, ED, SC, TEC, PS, EDP, NN, NZ, DNU, NDX, PPA, GZ, DU, MC, MCD, FA, MCF, AOX, DN, TP, CN, HCA, HCN, EA, ZD, SM

Table 10: Detergents with a "Low" output (BCA Assay)

No.	c [%]	AZ	SDN	PZ	MZ	RY
1	1.000	27.3	26.0	23.1	20.3	19.8
2	0.500	15.8	14.1	13.2	11.6	12.7
3	0.250	8.5	7.5	7.4	5.9	7.3
4	0.125	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
5	0.063	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
6	0.031	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
7	0.016	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
8	0.008	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
9	0.004	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
10	0.002	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ
11	0.001	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ