Comparability of oceanic nutrient data: Methods of certified reference material (CRM) production and certification for reference materials for nutrients in seawater (RMNS).

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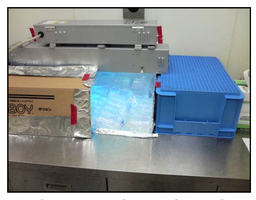
Abstract

KANSO CO., LTD. (KANSO) started the production of reference materials for nutrients in seawater (RMNS) in 2003. RMNSs were used in the 2003, 2006, 2008, and 2012 global inter-laboratory comparison studies conducted by Meteorological Research Institute (MRI, Japan), and a global inter-comparison study co-organized by the International Ocean Carbon Coordination Project (IOCCP) and Japan Agency for Marine-Earth Science and Technology (JAMSTEC) in 2015. The GO-SHIP nutrients measurement manual published in 2010 suggested KANSO's RMNS as one of the candidates to be used as common reference materials for all laboratories to improve the comparability of oceanic nutrients data set [1]. RMNS is produced by only natural seawater. The production method involves filtration of collected seawater and autoclave in a stainless-steel reaction chamber of which inside is electro-polished. After the seawater has been autoclaved, 90-mL aliquots are transferred into UV-sterilized polypropylene bottles in a clean room. The bottles are hermetically sealed in aluminum-film bag which has excellent barrier properties for suppressing long-term water evaporation. Nutrient concentration levels of RMNS range from low nutrient seawater in subtropical surface water to highest deep Pacific seawater. A range of nutrient levels is achieved by mixing collected natural seawaters of different nutrient concentrations at various mixing ratio [2,3,4,5,6]. A 320-L stainless-steel reaction chamber is used to autoclave natural seawater which produces 2500 bottles (ca. 90-mL aliquot) per one lot. The initial homogeneities of high-nutrient RMNS lots are less than 0.2 % (RSD). The stability of 4 determinants (nitrate, nitrite, phosphate and silicate) is guaranteed up to 7 years [7]. In 2011, KANSO was accredited as a certified reference material (CRM) producer under the Accreditation System of National Institute of Technology and Evaluation (ASNITE) program (accreditation number: ASNITE 0052 R). KANSO also meets the requirements of ISO Guide 34: 2009 (JIS Q 0034: 2012) and ISO/IEC 17025: 2005 (JIS Q 17025: 2005) for tests and measurements associated with assigning property values to certified reference materials. From 2011, the assigned certified values were the measured results of 120 randomly selected bottles by KANSO. Since 2015, the certified values have become the arithmetic means of independently obtained concentrations of 30 bottles each measured by JAMSTEC and KANSO. In this poster shows the production method of KANSO CRM and the results of independently measured nutrient concentrations by JAMSTEC and KANSO for certification of existing lots. The results show that two values agreed within measured expanded uncertainty (k = 2) for 9 lots produced. With more reliability in the measured values, the global use of KANSO CRMs is expected to improve comparability of seawater nutrient measurements.

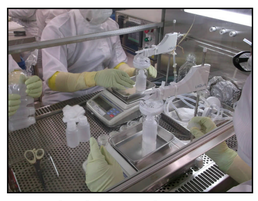
RMNS production procedure [8]

Source seawater is filtered (0.45 µm)

Mixed seawater is autoclaved twice in a 320-L stainless-steel reaction chamber at 120ºC for 2h



Polypropylene bottles (100 mL) are UV sterilized



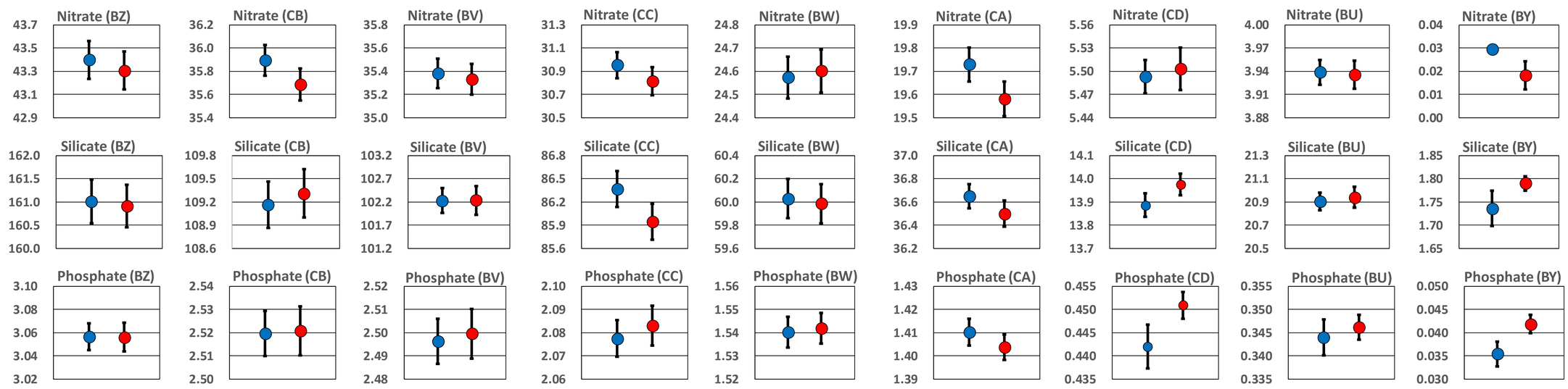
Bottled in a clean room (ca. 90-ml aliquot per bottle)

Packaged into hermetically-sealed aluminum-film bag

KANSO CRM (RMNS) Maximum of 2500 bottles produced per one lot

Certification

From 2015, to increase reliability in the certified values, the values have become the arithmetic means of independently obtained concentrations by JAMSTEC and KANSO.
Figure 1 is the results of independently obtained measurements for 9 RMNS lots. The values were obtained by measuring 30 bottles in duplicates using gas-segmented continuous flow analyzer (QuAAtro 2-HR, SEAL Analytical, UK).



●: KANSO Measured Value (n=30) and Uncertainty (k=1) ●: JAMSTEC Measured Value (n=30) and Uncertainty (k=1) RMNS lot names are in parentheses

Figure 1 Independently obtained nitrate, silicate, and phosphate concentrations by JAMSTEC and KANSO

Validity of certification

When conducting measurement for certification, KANSO CRMs and NMIJ CRMs were also measured. Table 1 shows the values are in good agreement and are within expanded uncertainty (k = 3) of each certified values.

Table 1 KANSO CRMs and NMIJ CRMs measured results

● **Nitrate** Unit: µmol/kg

Measured Lot	Measured Lab	Measured Value (n =30)	Measured Uncertainty (k =1)	BY	NMIJ CRM 7601-a	BU	NMIJ CRM 7602-a	CA	BW	BV	CB	NMIJ CRM 7603-a	BZ
BU	KANSO	**3.939**	**0.016**	*0.031*	*0.028*	3.924	15.50	19.61	24.59	35.39		44.20	43.49
	JAMSTEC	**3.936**	**0.018**	*0.005*	*0.010*	3.924	15.50	19.59	24.52	35.21		44.12	43.29
BV	KANSO	**35.38**	**0.13**	*0.027*	*0.030*	3.938	15.54	19.66	24.63	35.41		44.30	43.51
	JAMSTEC	**35.33**	**0.13**	*0.019*	*0.016*	3.940	15.49	19.56	24.51	35.29		44.17	43.37
BW	KANSO	**24.57**	**0.09**	*0.027*	*0.028*	3.923	15.51	19.60	24.60	35.33		44.22	43.43
	JAMSTEC	**24.60**	**0.09**	*0.037*	*0.015*	3.928	15.53	19.58	24.55	35.30		44.19	43.40
BY	KANSO	***0.030***	***0.001***	*0.025*	*0.027*	3.929	15.50	19.61	24.59	35.36		44.25	43.44
	JAMSTEC	***0.018***	***0.006***	*0.013*	*0.014*	3.909	15.45	19.50	24.47	35.21		44.12	43.32
BZ	KANSO	**43.40**	**0.16**	*0.033*	*0.039*	3.945	15.51	19.64	24.58	35.39		44.23	43.46
	JAMSTEC	**43.31**	**0.16**	*0.032*	*0.017*	3.928	15.49	19.57	24.50	35.20		44.06	43.34
CA	KANSO	**19.73**	**0.07**	*0.040*	*0.040*	3.973	15.57	19.72	24.68	35.47		44.32	43.58
	JAMSTEC	**19.58**	**0.08**	*0.010*	*-0.003*	3.911	15.50	19.55	24.52	35.28		44.14	43.34
CB	KANSO	**35.90**	**0.13**	*0.034*	*0.025*	3.919	15.53	19.66	24.61	35.40		44.23	43.46
	JAMSTEC	**35.69**	**0.14**	*0.047*	*0.041*	3.916	15.43	19.48	24.44	35.21		44.12	43.27
CC	KANSO	**30.95**	**0.11**	*0.031*	*0.023*	3.937	15.51	19.64	24.59	35.36	35.82	44.19	43.45
	JAMSTEC	**30.81**	**0.12**	*0.020*	*0.022*	3.926	15.49	19.50	24.55	35.25	35.74	44.19	43.26
CD	KANSO	**5.493**	**0.022**	*0.029*	*0.032*	3.958	15.52	19.63	24.60	35.34	35.81	44.23	43.41
	JAMSTEC	**5.503**	**0.028**	*0.027*	*0.023*	3.943	15.49	19.59	24.57	35.29	35.82	44.29	43.47
Average				***0.027***	***0.024***	**3.932**	**15.50**	**19.59**	**24.56**	**35.32**	**35.80**	**44.20**	**43.40**
Certified Value				***0.024***	***0.015***	**3.937**	**15.30**	**19.66**	**24.59**	**35.36**	**35.79**	**44.50**	**43.35**
Expanded Uncertainty (k =2)				***0.019***	***0.0081***	**0.051**	**0.48**	**0.15**	**0.20**	**0.35**	**0.27**	**0.58**	**0.33**

● **Silicate** Unit: µmol/kg

Measured Lot	Measured Lab	Measured Value (n =30)	Measured Uncertainty (k =1)	BY	NMIJ CRM 7601-a	BU	NMIJ CRM 7602-a	CA	BW	BV	CB	NMIJ CRM 7603-a	BZ
BU	KANSO	**20.91**	**0.08**	1.717	1.268	20.92	30.26	36.63	60.09	102.3		145.8	161.1
	JAMSTEC	**20.94**	**0.09**	1.653	1.186	20.86	30.24	36.60	59.95	102.1		145.8	160.9
BV	KANSO	**102.2**	**0.3**	1.765	1.311	20.99	30.29	36.72	60.08	102.3		146.1	161.0
	JAMSTEC	**102.2**	**0.3**	1.764	1.260	20.88	30.15	36.53	59.86	102.1		145.9	160.8
BW	KANSO	**60.03**	**0.17**	1.717	1.254	20.98	30.30	36.69	60.16	102.3		146.0	161.0
	JAMSTEC	**59.99**	**0.17**	1.718	1.203	20.82	30.20	36.50	59.87	102.0		145.7	160.7
BY	KANSO	**1.736**	**0.038**	1.741	1.275	20.90	30.19	36.53	59.99	102.2		145.8	160.9
	JAMSTEC	**1.790**	**0.015**	1.725	1.278	20.84	30.17	36.47	59.89	102.0		145.6	160.8
BZ	KANSO	**161.0**	**0.5**	1.744	1.286	20.97	30.31	36.74	60.14	102.4		146.0	161.3
	JAMSTEC	**160.9**	**0.5**	1.702	1.207	20.83	30.15	36.52	59.88	101.9		145.5	161.0
CA	KANSO	**36.65**	**0.10**	1.729	1.280	20.97	30.28	36.71	60.14	102.4		146.1	161.3
	JAMSTEC	**36.50**	**0.11**	1.639	1.133	20.68	30.03	36.36	59.69	101.9		145.6	160.5
CB	KANSO	**109.2**	**0.3**	1.718	1.260	21.00	30.36	36.76	60.27	102.4		146.2	161.3
	JAMSTEC	**109.3**	**0.3**	1.768	1.296	20.83	30.13	36.38	59.73	102.1		145.7	160.6
CC	KANSO	**86.37**	**0.23**	1.664	1.188	20.90	30.23	36.66	60.10	102.5	109.6	146.1	161.3
	JAMSTEC	**85.94**	**0.23**	1.761	1.302	20.88	30.27	36.56	59.91	102.0	109.1	145.7	160.7
CD	KANSO	**13.89**	**0.05**	1.650	1.187	20.85	30.14	36.56	59.95	102.3	109.4	146.1	161.1
	JAMSTEC	**13.97**	**0.05**	1.797	1.353	20.95	30.27	36.61	59.99	102.1	109.1	145.6	160.7
Average				**1.721**	**1.251**	**20.89**	**30.22**	**36.58**	**59.98**	**102.2**	**109.3**	**145.9**	**160.9**
Certified Value				**1.763**	**1.280**	**20.92**	**29.80**	**36.58**	**60.01**	**102.2**	**109.2**	**147.1**	**161.0**
Expanded Uncertainty (k =2)				**0.063**	**0.11**	**0.49**	**0.64**	**0.22**	**0.42**	**1.1**	**0.62**	**2.2**	**0.93**

● **Phosphate** Unit: µmol/kg

Measured Lot	Measured Lab	Measured Value (n =30)	Measured Uncertainty (k =1)	BY	NMIJ CRM 7601-a	BU	NMIJ CRM 7602-a	CA	BW	BV	CB	NMIJ CRM 7603-a	BZ
BU	KANSO	**0.344**	**0.004**	*0.035*	*0.042*	0.348	1.109	1.417	1.548	2.496		3.057	3.060
	JAMSTEC	**0.346**	**0.003**	*0.031*	*0.044*	0.344	1.104	1.409	1.538	2.482		3.039	3.049
BV	KANSO	**2.496**	**0.010**	*0.032*	*0.042*	0.343	1.105	1.415	1.543	2.494		3.053	3.059
	JAMSTEC	**2.500**	**0.011**	*0.046*	*0.059*	0.352	1.109	1.416	1.543	2.497		3.060	3.062
BW	KANSO	**1.540**	**0.007**	*0.040*	*0.052*	0.350	1.100	1.414	1.543	2.495		3.055	3.063
	JAMSTEC	**1.542**	**0.007**	*0.042*	*0.061*	0.352	1.109	1.412	1.543	2.485		3.042	3.054
BY	KANSO	***0.035***	***0.003***	*0.034*	*0.038*	0.344	1.099	1.404	1.534	2.486		3.045	3.053
	JAMSTEC	***0.042***	***0.002***	*0.039*	*0.055*	0.352	1.111	1.414	1.545	2.492		3.052	3.066
BZ	KANSO	**3.057**	**0.012**	*0.031*	*0.037*	0.337	1.102	1.412	1.539	2.489		3.053	3.061
	JAMSTEC	**3.056**	**0.012**	*0.039*	*0.054*	0.345	1.105	1.410	1.541	2.486		3.043	3.053
CA	KANSO	**1.410**	**0.006**	*0.034*	*0.046*	0.343	1.103	1.410	1.540	2.496		3.057	3.065
	JAMSTEC	**1.404**	**0.006**	*0.031*	*0.044*	0.341	1.098	1.401	1.531	2.477		3.037	3.044
CB	KANSO	**2.520**	**0.010**	*0.034*	*0.043*	0.347	1.109	1.418	1.548	2.503		3.060	3.071
	JAMSTEC	**2.521**	**0.011**	*0.036*	*0.054*	0.348	1.107	1.410	1.541	2.492		3.046	3.054
CC	KANSO	**2.078**	**0.008**	*0.035*	*0.039*	0.343	1.100	1.408	1.537	2.488	2.521	3.048	3.058
	JAMSTEC	**2.083**	**0.009**	*0.046*	*0.062*	0.356	1.111	1.409	1.546	2.489	2.519	3.047	3.068
CD	KANSO	**0.442**	**0.005**	*0.036*	*0.036*	0.346	1.100	1.409	1.537	2.491	2.520	3.050	3.061
	JAMSTEC	**0.451**	**0.003**	*0.042*	*0.052*	0.354	1.110	1.413	1.544	2.489	2.516	3.052	3.062
Average				***0.037***	***0.048***	**0.347**	**1.105**	**1.411**	**1.541**	**2.490**	**2.519**	**3.050**	**3.059**
Certified Value				***0.039***	***0.038***	**0.345**	***1.116***	**1.407**	**1.541**	**2.498**	**2.520**	***3.070***	**3.056**
Expanded Uncertainty (k =2)				***0.010***	***0.0053***	**0.0085**	***0.116***	**0.014**	**0.014**	**0.023**	**0.022**	***0.053***	**0.033**

Italic values are information values.

Measured Uncertainty = $(u_{JCSS}^2 + u_{chare}^2)^{1/2}$

Uncertainty calculation

Uncertainties of certified values were calculated using equation 1. Each uncertainty components is described in Table 2. As an example, calculated results for Lot CD nitrate is shown. Major uncertainty components will differ based on the type of determinands and concentration measured for certification.

Equation 1 Calculation formula for uncertainty determination

$$u_{CRM}^2 = u_{JCSS}^2 + (u_{char1}^2 + u_{char2}^2)/2 + u_{bb}^2 + u_{lts}^2 + u_{sts}^2$$

$$u_{char}^2 = u_{standard}^2 + u_{red}^2 + u_{NO2}^2 + u_{clib}^2 + u_{repeat}^2 + u_{density}^2$$

Table 2 Uncertainty of Nitrate (Lot CD: 5.50±0.05 µmol/kg)

Uncertainty of factor		Uncertainty (%)
u_{JCSS}	Uncertainty of JCSS concentration (Nitrate ion standard solution, 1000 mg/L)	0.35
u_{char1}	KANSO standard uncertainty due to characterization	0.18
$u_{standard1}$	KANSO standard uncertainty due to calibration solution concentration	0.13
u_{red1}	KANSO standard uncertainty due to rate of reduction	Insignificant
u_{NO2_1}	KANSO standard uncertainty due to nitrite concentration	Insignificant
u_{clib1}	KANSO standard uncertainty due to calibration curve	0.072
$u_{repeat1}$	KANSO standard uncertainty due to within-bottle	0.11
$u_{density1}$	KANSO standard uncertainty due to density conversion	0.018
u_{char2}	JAMSTEC standard uncertainty due to characterization	0.19
$u_{standard2}$	JAMSTEC standard uncertainty due to calibration solution concentration	0.093
u_{red2}	JAMSTEC standard uncertainty due to rate of reduction	0.015
u_{NO2_2}	JAMSTEC standard uncertainty due to nitrite concentration	0.039
u_{clib2}	JAMSTEC standard uncertainty due to calibration curve	0.034
$u_{repeat2}$	JAMSTEC standard uncertainty due to within-bottle	0.16
$u_{density2}$	JAMSTEC standard uncertainty due to density conversion	0.018
u_{bb}	Standard uncertainty due to between-bottle homogeneity	0.056
u_{lts}	Standard uncertainty due to long-term stabillty	0.020
u_{sts}	Standard uncertainty due to short-term stabillty	Insignificant
u_{CRM}	Standard uncertainty of a property value	0.40

Conclusion

For the certification conducted from 2015, independently obtained measured results by JAMSTEC and KANSO agreed within measured expanded uncertainty (k = 2) for 9 lots produced.
In addition, NMIJ CRMs and KANSO CRMs measured during the certification were also all within expanded uncertainty (k = 3) of certified values. With more reliability in the measured values, the global use of KANSO CRMs is expected to improve comparability of seawater nutrient measurements. We will continue to consider ways to minimize uncertainty to better monitor stability and quality of KANSO CRMs.

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Announcement: Company name has changed to KANSO CO.,LTD. (Former name: The General Environmental Technos Co., Ltd; effective June 29, 2016).