AutoAnalyzer Applications

Method No. G-173-96 Rev. 9 (Multitest MT18)

Nitrite in Water and Seawater

Ranges: 0 - 0.3 to 0 - 6 µmol/L (0 - 4.2 to 0 - 84 µg/L as N)

and 0 - 6 to 0 - 50 µmol/L (0 - 84 to 0 - 700 µg/Las N)

Description

This automated procedure for the determination of nitrite uses the procedure whereby the nitrite ion reacts with sulfanilamide under acidic conditions to form a diazo compound. This compound then couples with N-1-naphthylethylenediamine dihydrochloride to form a reddish-purple azo dye.

Hardware: 37°C heating bath (5.37 mL)
Pump tubes: 5 + 2 air + sampler wash
Multitest: Phenol, Phosphate, Total Phosphorus in Kjeldahl digests

Performance data using synthetic seawater standards

Test conditions: range: 0 - 2 µmol/L and 0 - 25 µmol/L, AA3 colorimeter with 10 mm flowcell and lamp

	Sample A	**Sample B**
	0 - 2 µmol/L	**0 - 25 µmol/L**
Pump tube	blu/blu	orn/yel
Sampling rate	60/hr	60/hr
Sample: wash ratio	4:1	4:1
Sensitivity: Extinction at 2 µmol/L/25 µmol/L as N	0.07	0.09
Reagent Absorbance	0.01	0.01
Coefficient of Variation (10 replicates at 50 %)	0.20 %	
Pooled Standard Deviation (25 randomised at 5 levels)	0.003 µmol/L	
Linear Correlation Coefficient	1.000	
Detection Limit (determined according to EPA procedure pt. 136, app. B)	0.003 µmol/L	
Detection Limit in lowest range (0-0.3 µmol/L; see operating note 12)	0.0015 µmol/L	

Note: the above performance specifications were developed with the exclusive use of genuine SEAL Analytical parts and consumables.

REAGENTS

Unless otherwise specified all chemicals should be of ACS grade or equivalent.

LIST OF RAW MATERIALS

	safety classification
Brij-35, 30% Solution	--
N-1-Naphthylethylenediamine dihydrochloride, $C_{12}H_{14}N_2.2HCl$	harmful
Phosphoric acid, conc., H_3PO_4	corrosive
Sodium chloride, NaCl	--
Sodium hydrogen carbonate, $NaHCO_3$	--
Sodium nitrite, $NaNO_2$	toxic
Sulfanilamide, $C_6H_8N_2O_2S$	--

Low-nutrient seawater: See operating note 2.

REAGENT MAKE-UP

Prepare reagents with distilled water or deionized water. Vacuum filter reagents through filter with pore size 0.5 µm or less for best results.

SYSTEM WASH SOLUTION and DILUENT

Brij-35, 30% solution	6 mL
DI water,	to 1000 mL

Add 6 mL of Brij-35, 30% solution, to 1000 mL of DI water.

SYNTHETIC SEAWATER (see operating notes 1 and 2)

Sodium chloride	35 g
Sodium hydrogen carbonate	0.2 g
DI water,	to 1000 mL

Dissolve 35 g of sodium chloride and 0.2 g of sodium hydrogen carbonate in about 800 mL of DI water. Dilute to 1000 mL with DI water.

COLOUR REAGENT

Sulfanilamide	10.0 g
Phosphoric acid, conc.	100 mL
N-1-Naphthylethylenediamine dihydrochloride	0.5 g
DI water,	to 1000 mL
Brij-35, 30% solution	4.0 mL

To approximately 750 mL of DI water add 100 mL concentrated phosphoric acid and 10.0 g of sulfanilamide. Dissolve completely. (Heat if necessary.) Add 0.5 g of N-1-naphthylethylenediamine dihydrochloride and dissolve. Dilute to 1000 mL with DI water. Add 4.0 mL Brij-35. Store in a cold, dark place. Stability: one month.

SPECIAL WASH SOLUTION

Use sodium hypochlorite solution diluted 1:5 with DI water.

STANDARDS

STOCK STANDARD A, 100 mg N/L

Sodium nitrite	0.493 g
DI water,	to 1000 mL

Dry about 0.6 g of sodium nitrite at 105°C for 2 hours. Dissolve 0.493 g of sodium nitrite in DI water and dilute to 1000 mL. Store in a dark bottle.

WORKING STANDARDS

Prepare working standards with sampler solution as required (see operating note 1 and 2).

OPERATING NOTES

1. For seawater analysis use sampler wash solution containing sodium chloride and sodium hydrogen carbonate if the method will be run at 60 samples per hour. For water and wastewater analysis use only DI water. It is possible to use DI sampler wash solution for seawater analysis, but the sampling rate will need to be reduced because there will be a disturbance at the beginning and end of each peak. The effect of this on peak reading is eliminated with *AA*CE software.

2. For the most accurate results, the standard diluent should have the same matrix as the samples. Therefore, use artificial seawater or low-nutrient seawater for seawater analysis. To avoid errors from silicate content in the inorganic salts used for artificial seawater, we recommend using a zero calibration standard of low-nutrient seawater of known low concentration. This is obtainable from Ocean Scientific International, Station Road, Petersfield, Hampshire, England GU32 3ET. Fax +44 1730 265011.

3. **Recommended procedures for best performance when analyzing low concentrations**
   - Pure water may be double distilled (DD) water or deionized (DI) water. In the case of DDW, the analyst must be careful to avoid contamination with silicic acid from dissolution of glass.
   - For accurate low-level work, all glassware used for making reagents should be rinsed with 10% hydrochloric acid followed by thorough rinsing with DI water two or more times. Store flasks “shaken dry” and capped. Regular cleaning of storage containers reduces variances in analytical results. Do not wash the glassware in a washer or with any kind of detergent.
   - Sample cups must be perfectly clean. For low-level work, fill sample cups with 10% hydrochloric acid and leave standing for at least 15 min. Then rinse the sample cups twice with DI water followed by two rinses with sample or standard solution.
   - Sample storage or transport containers may be made of any of several plastics. High density polyethylene or polypropylene bottles are very acceptable. Glass containers of any kind are not acceptable. Any glass contaminates the samples with silicic acid. Sample containers must be rinsed at least twice with sample before filling.
   - Skin contact must be avoided with anything which will touch the reagents and samples. Ammonia contamination of the air must be avoided (e.g. by smoking, farmyard, industrial smoke or vapour, other reagents).
   - The laboratory temperature should be reasonably stable, with no strong air currents around analyzer. Run the system with the manifold cover in place.
   - All chemicals should be of very high purity. Old and/or contaminated SDS will cause carryover, drift and noise. Final working standards are best prepared using natural artificial seawater of low nutrient content (see operating note 2).
   - The prepared reagents should be degassed by vacuum membrane filtration for best performance. Filter with a pore size of 0.5 µm or less should be used. The reagents, pure water and standards should be protected from atmospheric contamination.
   - Samples should be measured as soon as possible after sampling.

- Rinse the manifold according to operating note 4. Rinse wash receptacle each day by pumping baseline reagents for 15 minutes before starting a run. Clean the wash receptacle once a month with hypochlorite solution.
- The volume between the air valve and the injection fitting should be minimal, using 0.015" polyethylene tubing cut as short as possible. The joints between glass parts must be perfect without gaps.
- A regular bubble pattern is necessary for low noise. If the bubble pattern is irregular, check that all plastic tubing is correctly wetted (bubble shape round at front and back. After replacing the pump tubes or parts of the manifold, pump 1M NaOH through all tubes for 15 minutes. (see also operating note 11).
- If running only in the lowest range, connect the colour reagent to the line for sample B and tie off the line for the colour reagent. The baseline noise, and thus the detection limit, can then be further reduced by diluting the reagents by a factor of 2 or even 5. The linearity of the used range must be checked.

4. **Manifold cleaning procedure:**
Every day ⇨ pump system wash solution through all reagent lines.
Once a month ⇨ pump for 20 min. special wash solution (hypochlorite) through the system and the sample line, then 30 min. system wash solution.

5. If dual-range is not needed, remove sample line B and tie off or remove the T-piece.

6. Pump DI water through the sample line which is not connected to the sample probe.

7. The connection between the cartridge and the colorimeter should be made of glass or Kel-F in order to reduce the carryover.

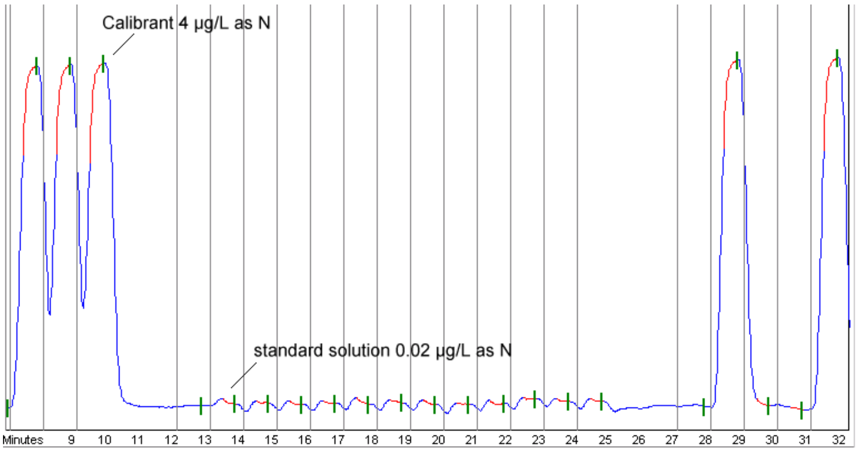
8. **Brij-35**
Old or oxidised Brij-35 can cause poor peak shape and low recovery. Do not use ready-prepared 30 % solution after the expiry date, and keep the bottle closed.

9. **Additional performance data**

	Sample A	**Sample B**
Lag time	5 min.	6 min.
Carryover	0.2%	

11. Even flow and regular air/liquid distribution in the transmission tube from the debubbler after the first mixing coil to the pump is critical to correct method performance. Check for correct flow and that the tubing is wetted (the trailing edge of the bubbles must be rounded, not straight). If necessary, especially for new tubing, increase the concentration of surfactant to achieve correct wetting.

12. Run of determination of detection limit in lowest range. Samples: seawater. Sampler wash: DI water.



13. LED Photometer
    By the operation of the AA3 on research vessels it is recommend to use the LED photometer. The noise of the signal caused by vibration and movement of the ship is reduced compared to the lamp photometer. The special filter for the LED must be used. The filter from the lamp photometer can not be used for the LED.

    The performance data may change slightly by the use of the LED photometer.

14. If the bubble pattern out of the heating bath becomes irregular the size of the second air injection pump tube may be increased from blk/blk to orn/orn.

REVISIONS

Revision 1, February 1999
Added AA3 data; integrated flow diagram

Revision 2, March 2001
Added operating note 11

Revision 3, April 2001
Added glassware inside AA3 colorimeter

Revision 4, November 2001
Expanded notes, slight text changes, removed AAII data

Revision 5, February 2002
Added LED photometer, expanded notes

Revision 6, May 2002
Correction of the flowchart

Revision 7, December 2002
Changed pump tube air to blk/blk(0.32)

Revision 8, April 2005
Added note 14

Revision 9, April 2008
Cahnged logo

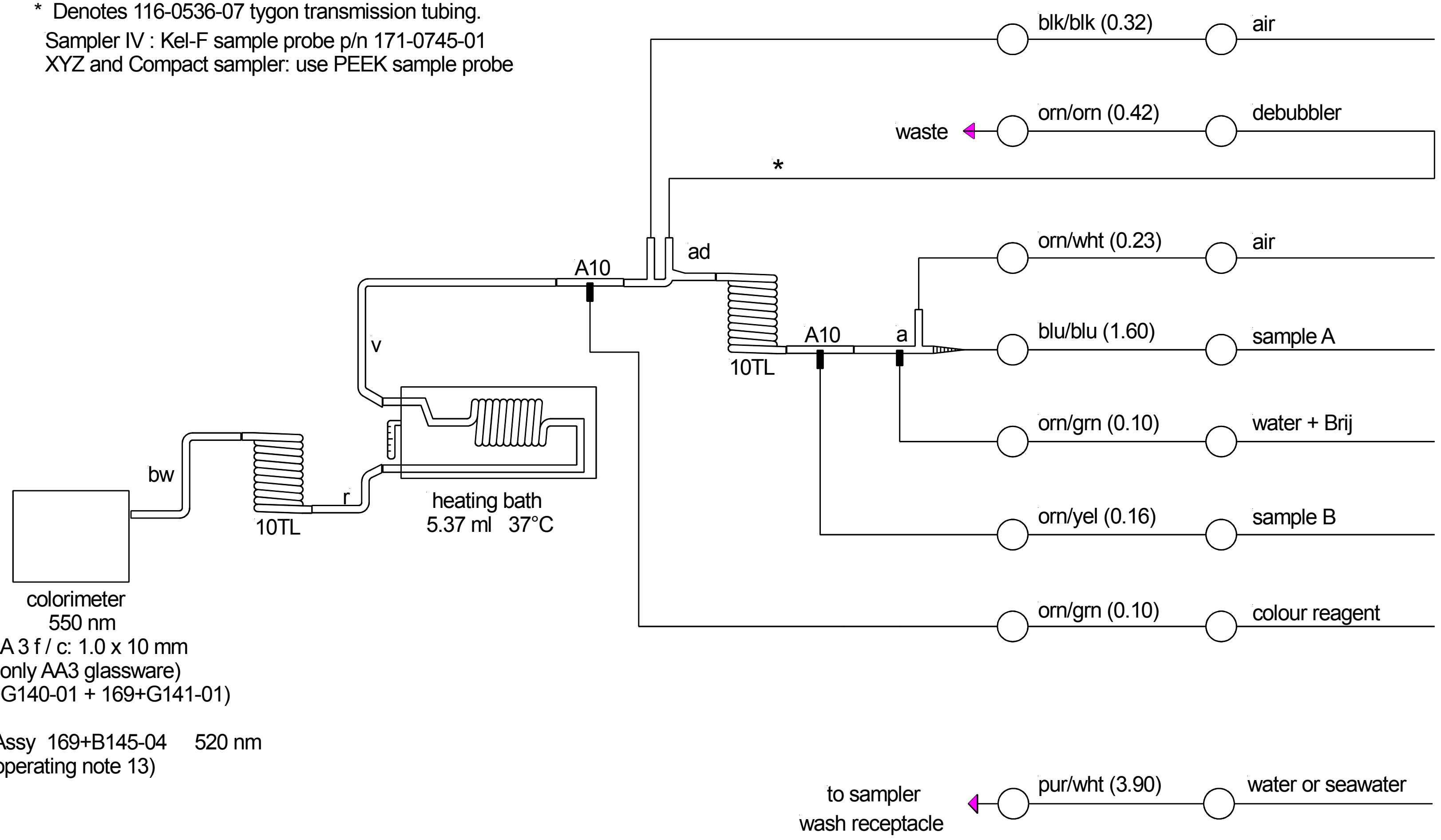
CONSUMABLES

The following estimated annual consumption rates are based on system operation 8 hours/day, 250 days/year.

Description	Legend	Part Number	Est. Annual Usage
BLK/BLK, 0.32 mL/min		116-0549-07	2 pkg./12
ORN/GRN, 0.10 mL/min		116-0549-04	2 pkg./12
ORN/YEL, 0.16 mL/min		116-0549-05	1 pkg./12
ORN/WHT, 0.23 mL/min		116-0549-06	2 pkg./12
ORN/ORN, 0.42 mL/min		116-0549-08	1 pkg./12
YEL/BLU, 1.40 mL/min		116-0549-19	1 pkg./12
BLU/BLU, 1.60 mL/min		116-0549-13	1 pkg./12
PUR/WHT, 3.90 mL/min		116-0549-18	1 pkg./12
Tubing air bar PharMed		117+0539-07	1 m
Polyethylene tubing 0.015” ID		562-2002-01	1 m
Polyethylene tubing 0.03" ID	*	562-2015-01	1 m
Tygon tubing		116-0536-07	1 m
Tygon tubing		116-0536-11	1 m
Tygon tubing		116-0536-16	1 m
Sample cups, plastic, 5 mL		171-0354-01	1 pkg./1000
Sample cups, plastic, 4 mL		127-0018-01	1 pkg./1000
Sample tubes, plastic, 8 mL		168-1000-01	1 pkg./2000
Sample tubes, plastic, 11 mL		168-1001-01	1 pkg./1600
Tubes for standards, 15.5 mL (XY2/3)		168-1004-01	1 pkg./100

SPARES			Recommended holding
AA3 Flowcell, 1.0 x 10 mm		169+B040-10	1 pc.
AA3 Filter Assy, 550 nm		165+B044-55	1 pc.
AA3 Heater assembly		169+B410-01	1 pc.
AA3 Coil, 5.37 mL		169+B441-01	1 pc.
AA3 Controller, 115V/230V		169+B430-01/02	1 pc.
LED Assy, 520 nm		169+B145-04	1 pc.
Thermometer 32-42°C		157-0283-01	1 pc.
Injection fitting, 3 pt.	a	116-0489-01	1 pc.
Glass coil, 10 turns left	10TL	157-0226-01	2 pcs.
Glass tubing	r	170-0193-01	1 pc.
Glass tubing	v	170-G014-01	1 pc.
Separator-phase 4 pt.	ad	021-G001-01	1 pc.
Connector T	A10	116-B034-01	2 pcs.
Glass tubing	bw	194-G003-05	1 pc.
AA3 colorimeter glassware		169+G140-01	1 pc.
AA3 colorimeter glassware		169+G141-01	1 pc.

NOTES : Figures in parentheses are flowrates in ml/min.
* Denotes 116-0536-07 tygon transmission tubing.
Sampler IV : Kel-F sample probe p/n 171-0745-01
XYZ and Compact sampler: use PEEK sample probe



LED Assy 169+B145-04 520 nm
(see operating note 13)

DRAWN	S.Giedigkeit	01.03.99	SYSTEM	AA3	PARAMETER	Nitrite
CHANGED	S.Giedigkeit	24.04.2008	METHOD NO. (8)	G-173-96	MATRIX	Water and seawater
RELEASED	A.Jorissen	24.04.2008	REMARK	Multitest MT18	RANGE	Sample A:0-0.3 to 0-6.0 µmol/l as N Sample B:0-6.0 to 0-50 µmol/l as N

SEAL Analytical

PROPRIETARY NOTE
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