
Stability of sealed-bag samples for off-line measurement of fractional exhaled nitric oxide

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Background: Fractional exhaled nitric oxide (FeNO) measurement is an index of airway eosinophilic inflammation and is primarily employed to diagnose bronchial asthma and assess airway inflammation. It can be measured with on- and off-line methods. In the latter, sample storage is possible; this is useful in Japan, because the number of facilities in which this instrument has been installed is limited.

Objective: We investigated the maximum period in which the samples continued to show stable values, as well as the influences of the storage environment.

Methods: Exhaled air samples were collected from 19 bronchial asthma patients (male/female: 8/11; mean age: 54.9 ± 15.6 years), and divided into 2 groups: a group with an FeNO level of less than 30 ppb and that with a level of 30 ppb or more. They were stored at 4 and 25°C to examine serial changes.

Results: The off-line-measured FeNO value was 33.7 ± 17.1 ppb, significantly lower than the on-line measured value (71.4 ± 47.7 ppb) ($P < .01$). In a group with an FeNO level of less than 30 ppb, temperature had no influence. However, in the other group, the period was prolonged to 72 hours when the samples were stored at 4°C. The samples were stable at 4°C for 24 hours, regardless of the initial measurements.

Conclusion: The duration of sample storage can be prolonged at 4°C. The standard FeNO measurement method is the on-line method, but the alternative use of the off-line method may contribute to the diagnosis and treatment of the patients.

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INTRODUCTION

The measurement of fractional exhaled nitric oxide (FeNO) is a simple and noninvasive test method reflecting eosinophilic inflammation of the airway.^{1,2} FeNO measurement is widely employed for the diagnosis and evaluation of bronchial asthma with chronic eosinophilic inflammation^{3,4} because it is less invasive compared with measurements of the rate and number of sputum eosinophils and cytokines and chemical mediators in the sputum supernatant and bronchioalveolar lavage fluid.^{5–7} In Japan, the number of facilities that have introduced FeNO measurement devices has been slowly increasing, but the spread is still insufficient because the devices are expensive and the test is not covered by health insurance.

On-line and off-line methods of FeNO measurement are available: expired gas is directly measured in the former, whereas it is collected in a bag exclusive for this purpose (Mylar bag, Sievers Instruments, Inc, Boulder, Colorado) and

then subjected to measurement in the latter. Although it is only an alternative to the on-line method, this off-line method may enable facilities without the measurement device to collect and send samples to those equipped to perform FeNO measurement.^{8–13}

Because of the measured values possibly altering over time, the American Thoracic Society and the European Respiratory Society (ATS/ERS) guidelines recommend measuring samples collected in Mylar bags within 12 hours.⁷ Only a few studies have been performed to investigate methods to prolong the storage time, and no consistent viewpoint concerning the storage conditions has been reached.¹⁴ In this study, we investigated the influence of the storage temperature on the stability of FeNO measurement samples for the off-line method.

METHODS

Subjects

Expired gas samples were collected from 19 patients with bronchial asthma (8 males and 11 females, mean age 54.9 ± 15.6 years, all nonsmokers) under treatment at the Department of Respiratory Medicine, Kawasaki Medical School Hospital. A definite diagnosis of bronchial asthma was made by the respiratory specialist at the outpatient clinic based on the respiratory function, bronchial responsiveness, blood tests, and treatment course according to the Global Initiative for Asthma guidelines.¹⁵ This study was approved by Kawa-

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saki Medical School Ethics Committee, and written informed consent was obtained from each patient.

Measurement of FeNO

Fractional exhaled nitric oxide was measured employing the off-line method, using Nitric Oxide Analyzer NOA 280i (Sievers Instruments, Inc). The measurement conditions of the off-line method were set following the ATS/ERS guidelines.⁷ From patients resting in a sitting position, expired gas after maximum inspiration was collected in a Mylar bag at an expiratory resistance of 5 cm H₂O or higher and expiratory flow rate of 0.35 L/sec (0.315–0.385 L/sec). The measurement was triplicated, and the mean was adopted. The first measurement was immediately performed (within 10 minutes), followed by repeated measurements every 24 hours to investigate the storage limit of the sample. The storage conditions at 4°C (refrigeration) and 25°C (room temperature) were compared. The storage limit was defined as the time at which the measured FeNO value became significantly different from that immediately after sampling.

Statistical Analysis

Statistical analysis was performed using Stat View 5.0 (SAS Institute Inc, Cary, North Carolina). The significance of changes in the measured FeNO value compared with that immediately after sampling was evaluated employing Student's *t* test, with *P* < .05 as significant.

RESULTS

Table 1 shows the background of the 19 patients. The off-line-measured FeNO value was 33.7 ± 17.1 ppb, significantly lower than the on-line-measured value (71.4 ± 47.7 ppb) (*P* < .01).

Using the method reported by Oshikata et al.,¹⁶ samples were divided into those containing FeNO at 30 ppb or higher and below 30 ppb. After the first measurement, the expired gas sample was divided into 2 portions, stored at 4 and 25°C, respectively, and measured every 24 hours. In the group with an initial value of less than 30 ppb (*n* = 9), significant changes in the measured value were noted within 48 hours at both temperatures. In the group with an initial value of 30 ppb or higher (*n* = 10), significant changes were noted within 24 hours at 25°C, but the time to the appearance of a significant

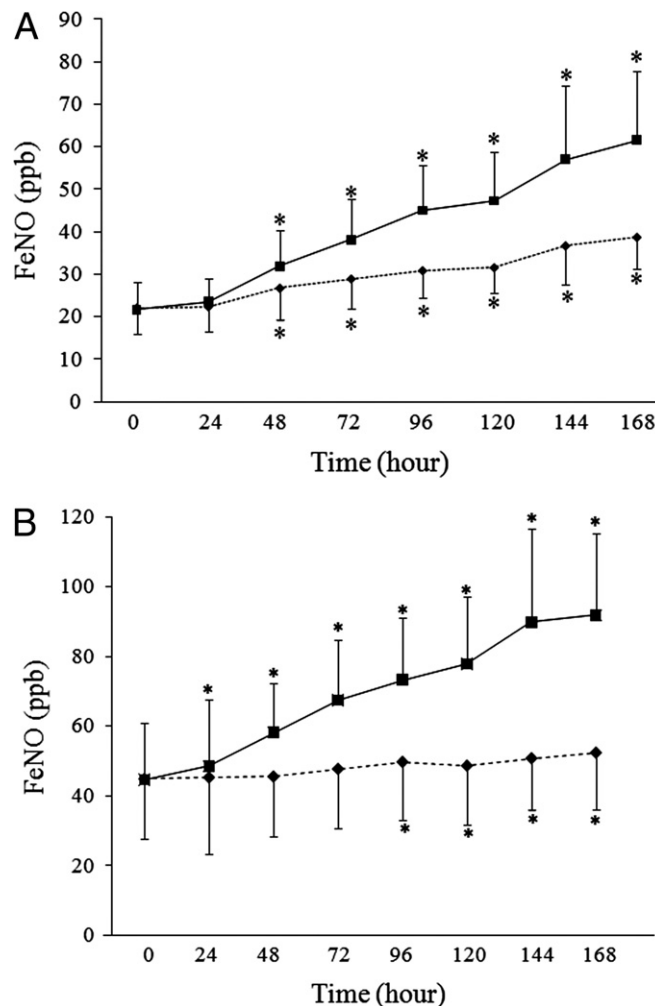


Figure 1. A, Temporal change of FeNO (low FeNO group <30 ppb). After the first measurement, the expired gas samples were divided into 2 portions, stored at 4 and 25°C, respectively, and measured every 24 hours. In the group with an initial value of less than 30 ppb (*n* = 9), significant changes in the measured value were noted within 48 hours at both temperatures. ♦, stored at 4°C; ■, stored at 25°C. **P* < .05 versus the first measured values. B, Temporal change of FeNO (low FeNO group ≥30 ppb). In the group with an initial value of 30 ppb or higher (*n* = 10), significant changes were noted within 24 hours at 25°C, but the time of the appearance of a significant change was delayed to within 96 hours by storage at 4°C. ♦, stored at 4°C; ■, stored at 25°C. **P* < .05 versus the first measured values.

Table 1. Patient Characteristics

Patient characteristics (<i>n</i> = 19)	
Sex (M/F)	8/11
Age (year)	54.9 ± 15.6
height (cm)	159 ± 7.36
FeNO <Online> (ppb)	71.4 ± 47.7 ^a
FeNO <Offline> (ppb)	33.7 ± 17.1
PEFR (L/min)	407 ± 150

FeNO, fractional exhaled nitric oxide; PEFR, peak expiratory flow rate.

^a Compared with FeNO <Offline>, *P* < .01.

change was delayed to within 96 hours on storage at 4°C (Fig 1).

DISCUSSION

On-line and off-line FeNO measurement methods have been reported, and the former is the global standard. Expired gas samples collected in Mylar bags are subjected to measurement in the off-line method, which has an advantage and disadvantages. The advantage is that samples can be collected at a site distant from the measurement device and collectively

measured,⁷ which is useful for facilities without the test equipment to employ FeNO measurement, such as those in Japan. As for the disadvantages, samples are likely to be influenced by storage conditions and time, and the measurement accuracy is inferior to that of immediate measurement of on-line collected expired gas at the collection site. Moreover, as shown in Table 1, even though measurement was performed immediately after sampling in a bag, the values tended to be lower than the on-line measured values. Several studies on the time limit of sample storage for the off-line method have been performed after the establishment of the ATS/ERS guidelines in 1999.^{9,14} The ATS/ERS guidelines were revised based on these in 2005, and recommend measurement within 12 hours after sampling.⁷ If this storage time limit can be prolonged by improving the storage conditions, more facilities may become able to employ the FeNO test, which may subsequently lead to improvement in the quality of life of these patients.

Regarding the storage temperature, a difference in the storage time limit between samples stored at the mean room (25°C) and cold room (4°C) temperatures in our hospital was investigated. When the initial measured value was less than 30 ppb, the value significantly changed after 24 to 48 hours, regardless of the storage temperature, and the shortest storage time limit was 24 hours. In contrast, when the initial value was 30 ppb or greater, the value significantly changed within 24 hours when stored at room temperature, but the time until the occurrence of a significant change was delayed to within 96 hours when stored in a cold room, showing that the low-temperature storage of samples containing FeNO at 30 ppb or higher prolonged the shortest storage time limit from less than 24 to 72 hours. This finding suggested that all groups of samples can be stored for at least 24 hours in cold conditions.

A limitation of this study was the small number of cases. We stopped collecting samples after a significant difference was observed when samples from 19 patients were compared. A possibility of influences of environmental factors other than temperature, such as humidity, on samples has been pointed out,¹⁴ but we did not investigate this because control of the environmental aspects other than temperature was difficult.

Although these problems were present, this study suggested that FeNO measurement becomes possible for facilities without the measurement equipment by transporting samples at a low temperature to equipped facilities. Measuring FeNO on-line as much as possible is desirable, but it is still expensive, and not all facilities can afford it. Employing the off-line method, FeNO measurement may become available

for many facilities and contribute to the diagnosis and treatment of patients.

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