Ideally, as suggested in the IFCC guideline, each laboratory should determine its own reference interval for $P_{50}$. Alternatively, an error analysis can be performed in the manner described here to estimate a lower bound for a 95% confidence interval.

References

Optimization of Nitric Oxide Oxidiuminescence Operating Conditions for Measurement of Plasma Nitrite and Nitrate, Ryon M. Bateman,1,2 Christopher G. Ellis,1,2 and David J. Freeman1,7 (1 Vascular Biology Program, Lawson Health Research Institute, London Health Sciences Centre, London, Ontario, N6B 1B8 Canada; Departments of 2 Medical Biophysics and 3 Medicine, University of Western Ontario, London, Ontario, N6A 5C1 Canada; * address correspondence to this author at: Department of Medicine, University of Western Ontario, University Hospital 339 Windermere Rd., London, Ontario, N6A 5A5 Canada; fax 519-663-3789, e-mail dfreeman@uwo.ca)

The oxidized nitric oxide (NO) metabolites nitrite (NO$_2^-$) and nitrate (NO$_3^-$) have been used as markers of NO synthase activity in several clinical and experimental studies including those investigating sepsis (1–4), septic shock (5), endotoxemia (6,7), cardiac transplant (8), hypertension (9), and cancer (10). Although the NO chemiluminescence methodology has been used to measure NO$_2^-$ and NO$_3^-$ (11–13), there are few reports evaluating the operating conditions of the assay.

The operating efficiency of the NO chemiluminescence system is governed by several variables: driving pressure and flow rate of the carrier gas (14, 15), which determine the degree of mixing in reducing solution and dispersion of NO in the carrier gas stream; chemiluminescent reaction chamber pressure ($P_{RC}$); and the selectivity (16), pH (11), temperature (13), and concentration of the reducing agents used to convert NO$_2^-$ and NO$_3^-$ to NO. Yang et al. (13) studied the efficiency of conversion of both NO$_2^-$ and NO$_3^-$ to NO using various reducing agents over a range of operating temperatures; however, no studies on the relationship between carrier gas flow rate and $P_{RC}$ have been reported.

The present study had three primary objectives: (a) to determine the optimal operating conditions for carrier gas flow rate and $P_{RC}$ to achieve maximum efficiency of the chemiluminescent response for both NO$_2^-$ and NO$_3^-$ determinations; (b) to determine the detection limit and linearity of NO$_2^-$ and NO$_3^-$ responses on the basis of these optimal operating conditions; and (c) to evaluate the recovery of NO$_2^-$ and NO$_3^-$ from plasma and deproteinized plasma under optimized operating conditions.

We purchased potassium iodide, sodium nitrite, sodium nitrate, and glacial acetic acid from Sigma, vanadium (III) trichloride, and hydrochloric acid from Aldrich Chemical Co., and helium and oxygen from Praxair.

The NO chemiluminescence system used in these experiments consisted of four main components: helium carrier gas (set at 35 psi), two in-parallel purge vessels with condensers connected by T-valves, a nitric oxide analyzer (NOA) with inlet valve and chemiluminescence reaction chamber (Sievers 270B, NO Chemiluminescence Analyzer, Sievers Instruments), and a vacuum pump (Edwards Pump) to draw the carrier gas into the reaction chamber. Helium carrier gas passed through a chemical trap (2 mol/L NaOH) before entry into the NOA to remove volatile acids. Connecting tubing was 3.2 mm i.d. (890 FEP; Nalge Nunc International). One purge vessel (10-mL capacity) was dedicated to KI reduction at room temperature, whereas the other (12 mL-capacity and fitted with a cold-water condenser) was dedicated to V(III) reduction at 90 °C. The helium flow rate into either purge vessel was controlled by a flow meter (Matheson Gas Products) placed upstream of the purge vessels. Operating pressure of the NO chemiluminescence reaction chamber was controlled by a needle valve at the entrance to the NOA. Inlet oxygen pressure, used to generate ozone, was 6 psi. In brief, NO$_2^-$ or NO$_3^-$ calibrators were injected (10 µL) into a purge vessel containing either KI or V(III) and converted to NO. NO was then stripped from the reducing medium by a helium carrier gas and transported, via the chemical trap, to the chemiluminescence reaction chamber where it reacted with ozone to generate the chemiluminescent signal as described previously (17).

Operating conditions were adjusted to evaluate efficiency of the NO chemiluminescent response at different gas flow rate and $P_{RC}$ combinations. Plots of NO chemiluminescence responses for various flow rates against $P_{RC}$ are called isoflow curves. The strength, concentration, and temperature of reducing agents used were established according to recommendations from the NOA manufacturer (18) and by Yang et al. (13). NO$_2^-$ was reduced to NO in KI solution (50 mg·1 mL$^{-1}$ of water + 4 mL$^{-1}$ of glacial acetic acid) at room temperature (22 °C) to mini-
mize foaming of the reducing solution. NO$_3^-$ was re-
duced to NO in V(III) (3.5 mL; 0.05 mol/L in 0.8 mol/L
HCl) at 90 °C.

Aqueous 30 μmol/L calibrator samples of NO$_2^-$ and
NO$_3^-$ were prepared by diluting NO$_2^-$ and NO$_3^-$ into
deonized water or fresh plasma obtained from heparin-
ized rat whole blood. Plasma samples were also depro-
teinized by acetonitrile (1:1 by volume) to limit potential
interference from hemoglobin (17). Supernatants were
collected by centrifugation (3000g for 3 min at 4 °C).

Kruskal–Wallis one-way ANOVA on ranks was used to
assess differences in the time of NO chemiluminescence
signal onset over various operating conditions (SigmaStat
2.0; SPSS Inc.). Dunnett’s method was used for pairwise
multiple comparison with the highest flow group (flow
rate, 126 mL/min) acting as the control group. Regression
analysis was performed with SigmaPlot 5.00 (SPSS Inc.
Chicago, IL). $P < 0.05$ was considered statistically signifi-
cant.

Isoflow curves of NO chemiluminescence responses
from 300 pmol of NO$_2^-$ and NO$_3^-$ in both aqueous
solution and plasma are depicted in Fig. 1. In all cases, for
a given flow rate, the maximum NO signal was depend-
ent on $P_{RC}$. As helium carrier gas flow rates increased,
from 42 to 126 mL/min, maximum NO signal response
was obtained at increasingly higher $P_{RC}$. These responses
demonstrated that the maximum NO signal was depend-
ent on both flow rate and $P_{RC}$.

The detection limit and linearity of the NO chemili-
uminescent response for aqueous NO$_2^-$ and NO$_3^-$ conversion
to NO at optimal low (flow rate, 42 mL/min; $P_{RC}$, 1133 Pa),
medium (flow rate, 69 mL/min; $P_{RC}$, 1100 Pa), and high
(flow rate, 126 mL/min; $P_{RC}$, 1100 Pa) flow rate states are
shown in Table 1. Both the detection limit and linear range
were dependent on operating conditions with the lowest
detection limit and greatest linear range being achieved at
a high flow rate. Although greater NO signals were
obtained at a low flow rate, detection limit was improved

Fig. 1. Isoflow profiles for aqueous and plasma NO$_2^-$ and NO$_3^-$ conversion to NO under various flow rate and $P_{RC}$ combinations.

Isoflow profiles for conversion of 300 pmol of NO$_2^-$ to NO in aqueous solution and plasma by KI in glacial acetic acid at 22 °C (A and B) and 300 pmol of NO$_3^-$ to
NO in aqueous and plasma solution by V(III) at 90 °C (C and D). Endogenous plasma background NO$_2^-$ and NO$_3^-$ responses were not subtracted from the sample NO chemiluminescent signal.
at a high flow rate because the signal-to-noise ratio was increased. Additionally, the onset of NO signal at the high flow rate (12.8 ± 0.2 s) decreased (P < 0.05) compared to low flow rate (29.4 ± 0.2 s). Under high flow rate conditions, recovery of nitrite (10–100 000 pmol) in plasma and deproteinized plasma ranged from 92.2% ± 6.8% to 100.2% ± 2.5% and 87.3% ± 5.5% to 111.7% ± 8.9%, respectively. Nitrate recovery (10–100 000 pmol) was similar (i.e., in plasma, 101% ± 2.3% to 96.1% ± 1.2%; in deproteinized plasma, 89% ± 1.6% to 101.7% ± 2.2%, respectively). Recovery at 1 pmol, although detectable, was not readily quantifiable electronically because of increased background signal. The decrease in sensitivity in plasma, relative to aqueous samples, was attributable to increased background signal in both plasma and deproteinized plasma.

Isolow flow curves show that NO chemiluminescent signal is dependent on both flow velocity of helium carrier gas and \( P_{RC} \). This is consistent with earlier reports that chemiluminescent signal is related to the rate of carrier gas flow (14, 15). Increasing the rate of the carrier gas into the purge vessel should increase the rate of mass transfer of NO from the liquid phase into the gas phase, thereby increasing the efficiency of the separation process and also decreasing the axial dispersion of the NO in the helium flow stream. However, the probability of a successful collision between NO and ozone within the reaction chamber may decrease because of decreased NO residence time, thereby reducing signal intensity; although, this is offset somewhat by higher \( P_{RC} \), despite the likelihood of enhanced nonradiative collisional quenching of the excited state of NO2 (NO2*) (18).

We determined the maximum NO chemiluminescent signal for all flow rates tested and observed that as flow rates increased, the maximum signal not only decreased, but occurred at higher \( P_{RC} \) (Fig. 1). This indicated a complex relationship among flow, \( P_{RC} \), and NO signal. Interestingly, this complex relationship is similar in some respects to the efficiency profile of gas and liquid chromatographic separations, where the maximum efficiency is related to the carrier gas velocity at which the theoretical plate height is minimized (19). At high flow rates, the detection limit was enhanced (Table 1) because of increased peak stability at low concentrations (i.e., electronic area integration was improved).

In conclusion, a range of carrier gas flow rates have been reported (100 mL/min of N2 to 200 mL/min of helium) for chemiluminescence-based analysis of NO2 and NO3 (11–13). On the basis of our results, we recommend the use of a high carrier gas/mobile phase flow rate at optimized \( P_{RC} \). Despite a reduction in NO chemiluminescent signal response, this increases sensitivity and linear response of the assay and has the additional benefit of decreasing analysis time.

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References


Interchangeability of Estimates of Day-to-Day Imprecision between Commercial Control Materials and Serum Pools, Xavier Fuentes-Arderiu* and Bernardino González-de-la-Presa (Servei de Bioquímica Clínica, Ciutat Sanitària i Universitaria de Bellvitge, L’Hospitalet de Llobregat, 08907 Catalonia, Spain; * author for correspondence: xfa@csub.scs.edu)

Frequently it is assumed that the precision of a measurement system observed using commercial control materials is the same as would be observed using human samples without additives. However, warnings that this assumption may be erroneous were published almost 20 years ago (1).

To provide more information regarding this obscure point of metrology applied to clinical laboratory science, we have studied the interchangeability of day-to-day imprecision for commercial control materials and serum pools (Table 1).

Measurements of hormones and troponin T were made with the Elecsys 2010 analyzer (Roche Diagnostics), and the remaining measurements were performed with the Hitachi 747 analyzer (Roche Diagnostics), according to the manufacturer’s protocols in both cases.

For each measurement procedure, day-to-day imprecisions were estimated simultaneously with Unassayed Chemistry Control 1 and Unassayed Chemistry Control 2 (unassayed reference materials 731 and 732, respectively; Bio-Rad) as control materials and two serum pools from routine patient samples having values near the values obtained in the control materials. The final values of control materials and pools are given in Table 1.

Each control material was reconstituted previously, and each serum pool was divided into 20 aliquots and stored at −20 °C until measurement. Daily, over 20 working days, one measurement of each analyte was carried out in each of the control materials and serum pools. When replicated results for each analyte were obtained, the corresponding variances and CVs representing day-to-day imprecision were estimated. All CVs are shown in Table 1.

To determine the interchangeability of the day-to-day imprecisions determined for the two types of materials, each pair of variances (corresponding to results obtained with the control material and the serum pool having similar values) was compared by the F-test. P <0.05 was regarded as significant. Results of these comparisons are shown in Table 1. These data suggest that the variances for the control materials and serum pools used in this study were interchangeable for measurements of cholesterol, phosphate, thyrotropin, thyroxine, free thyroxine, triglyceride, triiodothyronine, and urea; however, these variances across control materials and patient serum pools were not interchangeable for measurements of alanine aminotransferase, bilirubin, calcium, creatine kinase, creatininum, ferritin, glucose, or troponin T. In the case of measurements of aspartate aminotransferase, pancreatic α-amylase, and protein, interchangeability was dependent on concentration. For those tests that did not demonstrate interchangeability, day-to-day imprecision, in general, was lower with control materials. Hohnadel et al. (1) reported similar results for cholesterol, glucose, and urea.

The lack of interchangeability between commercial control materials and serum pools regarding day-to-day imprecision may have adverse consequences in monitoring patient results. In fact, the detection of a significant change between two consecutive results of an analyte in a patient requires knowledge of the day-to-day imprecision associated with patient results (2). Because estimations of day-to-day imprecision are usually made with control materials, a lack of interchangeability in these cases diminishes the reliability of criteria developed to detect significant changes. In addition, a lack of this type of interchangeability can lead to misestimation of the uncertainty of measurement of a patient’s results (3).