

1998

ARR26 - 3408



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Validation of Determination of Total Fluorine in Blood by Wickbold Torch with Direct Potentiometric Detection.

Principle:

Wickbold Torch (WT) is an analytical sample digestion method where the fluorine-containing sample is combusted by hydrogen/oxygen flame and converted to inorganic fluoride. Fluoride is condensed and absorbed in a small amount of water. The resulting solution is then analyzed by direct potentiometry using a fluoride ion-selective electrode (F-ISE) to obtain its concentration in the measured solution. This concentration is directly proportional to the total amount of fluorine being combusted by WT.

Procedure:

Validation of determination of Fluorine by WT/F-ISE in blood was performed in two steps:

1. Validation of potentiometric determination of fluoride by F-ISE.
2. Validation of determination of fluorine in blood by WT/F-ISE.

A. Validation of potentiometric determination of fluoride by F-ISE.

The following method was used for direct potentiometric determination of fluoride:

- 1.1 Calibration curve dE vs. $\log c_F$ was measured daily where dE is the difference between the F-ISE potential of 20 ppb F^- reference solution and the F^- standard solution. 20 ppb reference solution was measured immediately before each measured calibration standard. c_F is the concentration of F^- in the calibration standard. Calibration standards containing 30, 50, and 100 ppb F^- were used.
- 1.2 Performance of the F-ISE was evaluated daily based on the deviation of the calibration curve slope from the theoretical slope value (S). Considering that the F-ISE was operated in the non-linear portion of its response, the tolerance of $S \pm 5$ was allowed.

Where
$$S = \frac{RT}{F} \ln 10$$

Where R is universal gas constant, T is temperature in Kelvin, and F is Faraday's constant.

- 1.3 Validation samples for F-ISE measurement were prepared by dissolving the appropriate amount of dried NaF in MilliQ H_2O . This primary stock solution was serially diluted with water to produce appropriate concentrations of F^- in water. All F^- solutions (including those from samples) were transferred and stored in plastic (HDPE) bottles.
- 1.4 To evaluate the contribution of F-ISE measurement and the sample manipulation to the uncertainty of the final WT analytical results, simulated samples were treated the same way as the actual WT blood samples would be. Except, the burning process was not performed. That is, 1.5g of sample (recorded with analytical precision) was transferred to a plastic bottle to which the appropriate amount of NaAc buffer and F^-

spike was added. The volume of the solution was then increased to 150g with MilliQ water (to simulate the final volume after the WT burn). The amount of F⁻ spike added to each sample solution was calculated in a way that, after dilution of the solution to 150g, the contribution of F⁻ spike to the final F⁻ concentration would be exactly 20 ppb (the same as the concentration of the reference solution measured before each sample/standard). The system blank was simulated in the same way (0.0000g of sample was added to the simulated system blank bottle).

- 1.5 pH and ionic strength of each measured solution were kept constant by means of sodium acetate (NaAc) buffer added to each measured solution to produce a constant NaAc concentration in each measured solution. pH was kept at 5.
- 1.6 F-ISE was allowed to equilibrate with each measured solution for 10 minutes at a constant stirring rate. After each 10-min measurement the electrode was dipped for 6 seconds into a rinsing solution (stirring on during rinsing). Timing and stirring rate were controlled electronically by Titrino 751 potentiometric titrator system.

Results and Discussion

Accuracy, reproducibility, and operator to operator variability were evaluated for automated determination of F⁻ by F-ISE.

Accuracy was evaluated by means of relative error of a single determination of samples at 1, 5, 20, and 100 ppm F⁻ in water.

Reproducibility was evaluated at 1, 5, and 100 ppm concentration levels by analysis of multiple identically prepared samples (n = 10).

Operator to operator variability was evaluated by having two operators perform the above accuracy and reproducibility analyses independently.

Table I.

Accuracy evaluation of determination of F⁻ by F-ISE by two operators.

Actual F ⁻ concentration (ppm)	Operator I		Operator II	
	Determined F ⁻ concentration (ppm)	relative error (%)	Determined F ⁻ concentration (ppm)	relative error (%)
1	1.38	38 ^a	0.88	-12
5	5.23	4.8	5.10	2.0
20	21.7	8.4	19.9	-0.4
100	109	9.2	90.2	-9.8

^a measurement influenced by extensive exposure of F-ISE to water, disregard result (see text)

Table II.

Reproducibility evaluation of determination of F^- by F-ISE at 5 ppm by two operators.

Operator I ^a			Operator II ^b	
Actual F^- concentration (ppm)	Determined F^- concentration (ppm)	relative error (%)	Determined F^- concentration (ppm)	relative error (%)
5	5.57	11	5.10	2.0
5	5.39	7.7	4.95	-1.0
5	5.31	6.2	4.92	-1.6
5	5.65	12.9	4.90	-2.0
5	5.27	5.4	4.85	-2.9
5	5.24	4.7	5.06	1.1
5	5.16	3.1	4.84	-3.1
5	5.22	4.4	4.89	-2.1
5	5.05	1.1	4.83	-3.4
5	5.17	3.9	4.85	-2.9
average	5.31	6.1	4.92	2.2
st. dev.	0.18	3.7	0.09	0.8
%CV	3.47		1.87	

^a disposable graduated glassware used for volume transfers

^b volumetric glassware used for volume transfers

Table III.

Reproducibility evaluation of determination of F⁻ by F-ISE at 100 ppm by two operators.

Actual F ⁻ concentration (ppm)	Operator I		Operator II	
	Determined F ⁻ concentration (ppm)	relative error (%)	Determined F ⁻ concentration (ppm)	relative error (%)
100	106	6.2	105	5.3
100	97.1	-2.9	104	4.2
100	108	8.3	103	3.2
100	101	1.3	129 ^a	29 ^a
100	106	5.7	91	-8.7
100	109	8.8	105	5.2
100	103	3.3	108	7.8
100	103	2.5	103	2.8
100	110	9.6	105	5.1
100	105	5.1	107	7.3
average	105	5.4	104	5.5
st. dev.	3.84	2.9	4.90	2.0
%CV	3.66		4.73	

^a measurement influenced by extensive exposure of F-ISE to water, result excluded from average, st. dev., and %CV calculations (see text)

It can be seen from tables I, II, and III that the accuracy (as indicated by % relative deviation from the nominal concentration value) and precision (as indicated by standard deviation and %CV's of replicate measurements) of the F-ISE determination of F⁻ in water do not seem to be operator-dependent.

As indicated by the results summarized in Table II, the accuracy and precision of the method can be improved approximately two-fold when more accurate volumetric glassware is employed for solution preparation and volume transfers during the analysis. The accuracy and precision of the technique did not improve when higher F⁻ concentrations were analyzed as indicated by the standard deviation and %CV values in Table III compared to Table II. This is due to the fact that the F⁻ concentrations in the final simulated WT solutions resulting from 100 ppm samples had to be 20x diluted to fit to the calibration curve range. This additional step and more sample manipulation had adverse effect on the standard deviations and %CV's one would expect from higher concentration samples.

The results shown in Table III include one significant outlier. The results shown in Table I, II, and III were obtained when water was used as washing solution for F-ISE (see Procedure 1.6). If the electrode is allowed to rest in water (e.g. during,

loading/unloading the sample changer, titrator data entry, when the sample sequence is finished) for extended period of time (more than 30 seconds), the electrode will be entering the next sample solution polarized to very high potentials. Consequently, the 10-min equilibration time will not be sufficient for that solution. Since all data solutions are measured in pairs (20 ppb reference and an actual sample/standard), it is usually the 20 ppb reference measurement which gets affected. Therefore, the problem is easy to diagnose by comparing all the 20 ppb measurements for the day and correlating the way the analysis was done with any observed 20 ppb outlier. This situation occurred for one sample in Table I and one sample in Table III. Both of these results should be disregarded. In order to minimize this effect, 10 ppb F⁻ solution was used as wash solution for subsequent experiments.

It is necessary to note that a similar effect was observed (to a much smaller extent) for the 20 ppb reference measurement measured right after 100 ppb standard. This is due to the fact that 100 ppb standard (highest concentration standard) produces relatively low electrode potentials compared to the rest of the standards and samples. Therefore the next solution might require slightly longer equilibration time. It is critical for WT analysis that the 20 ppb reference corresponding to the system blank for a sample set is not influenced by this effect because all the results for the sample set depend on the value and accuracy of the measurement of system blank (and its 20 ppb reference solution). To avoid this situation, the standards should be measured in a descending order, that is 20, 100, 20, 50, and 20, 30 ppb (where the 20 ppb is the reference solution measured before each standard, see part A 1.1). When the standards are measured in this order, it is the 20 ppb reference corresponding to 50 ppb standard which will get slightly affected and not the system blank reference. More accurate WT data can be produced this way. The effect on 50 ppb measurement is minimized by means of linear regression ; performed on all three standards. This is also supported by the fact that the correlation coefficients of our calibration curves from multiple days were higher than 0.999 (frequently values higher than 0.9999 were observed).

Since the effects and observations discussed above could not be made until the results in Table I, II, and III were obtained, additional testing was performed at 1 ppm F⁻ levels. The results from these tests are summarized in Table IV. In this table, 0.5g sample size was used (to simulate the extent of dilution for real WT blood samples), and the changes discussed above were implemented (standards measured in descending order, 10 ppb F⁻ solution used as wash solution).

Table IV.

**Reproducibility evaluation of determination of F⁻ by F-ISE at 1 ppm
0.5g sample size**

Actual F ⁻ concentration (ppm)	Operator II	
	Determined F ⁻ concentration (ppm)	relative error (%)
1.0	1.13	13
1.0	1.12	12
1.0	0.98	-1.6
1.0	1.01	0.7
1.0	1.01	0.9
1.0	1.00	0.2
1.0	0.93	-7.1
1.0	0.92	-8.0
1.0	0.84	-16
1.0	0.87	-13
average	0.98	7.2
st. dev.	0.09	6.0
%CV	9.64	

B. Validation of determination of fluorine in blood by WT.

The method for determination of fluorine in whole blood by WT/F-ISE was evaluated in terms of accuracy, reproducibility, and carryover.

Accuracy of the determination was evaluated based on the % relative deviation of the results produced by the method compared to the targeted fluorine contents in the blank blood samples spiked with known amounts of fluorine.

Reproducibility/precision was evaluated based on multiple analyses of samples prepared at the same fluorine concentration level. Multiple analyses of the same sample were performed as well as analyses of identically prepared samples on different days.

Carryover evaluation was done by comparing the absolute fluorine amounts determined during system blank determination (before the start of any sample analysis) with the absolute fluorine amounts determined immediately after the analysis of high fluorine containing samples (approx. 25 and 100 ppm). For these determinations, 0.0000g of sample was burned and treated the same way as if it was a real blood sample.

All the blood for the WT validation was from male rats not treated with any special fluorinated diet. Fluorine spiked into blood was in the form of aqueous solution of ammonium perfluorooctanoate (abbreviated as C8). Appropriate amounts of solid C8 were weighed and dissolved in water to produce the targeted concentrations of fluorine in water/blood. All the sample preparation and standard C8 preparation was done on a weight/weight basis in order to ensure the best possible accuracy. Every final blood sample contained 90% of blood matrix and 10% of spike. Sample size of all blood samples processed through WT was 0.5g (measured with analytical precision).

Table V.

Accuracy evaluation of WT/F-ISE determination of F in whole rat blood at low concentration levels.

Actual F concentration (ppm)	Determined F concentration (ppm)	relative error (%)
0	-0.13	-
0.17	-0.20	-
0.55	0.70	27
1.02	1.07	4.9
1.02	0.84	-17
4.81	4.44	-7.7

Analysis by: Operator I

Apparatus: WT1

Table VI.

Accuracy and carryover evaluation of WT/F-ISE determination of F in whole rat blood at high concentration levels.

Actual F concentration (ppm)	Determined F concentration (ppm)	relative error (%)	System Blank F content (μg)
<i>sys. blank 1</i>			<i>0.750</i>
0	-0.03	-	
10.18	10.48	2.9	
25.42	23.64	-7.0	
<i>sys. blank 2</i>			<i>0.769</i>
101.36	96.04	-5.2	
<i>sys. blank 3</i>			<i>0.879</i>

Analysis by: Operator: I
Apparatus: WT1

Table VII.

Reproducibility evaluation of WT/F-ISE determination of F in whole rat blood at high concentration levels.

Actual F concentration (ppm)	Determined F concentration (ppm)	relative error (%)
23.46	23.33	-0.5
23.46	22.40	-4.5
23.46	21.60	-7.9
average	22.45	4.3
st. deviation	0.87	3.7
%CV	3.86	
Analysis by: Operator I		
Apparatus: WT1		
25.60	27.22	6.3
25.60	25.07	-2.1
25.60	26.10	2.0
average	26.13	3.4
st. deviation	1.07	2.5
%CV	4.11	
Analysis by: Operator II		
Apparatus: WT2		

Table VIII.

Reproducibility evaluation of WT/F-ISE determination of F in whole rat blood at low concentration levels.

Actual F concentration (ppm)	Determined F concentration (ppm)	relative error (%)
4.96	4.97	0.1
4.96	4.85	-2.3
4.96	4.91	-1.1
4.96	4.79	-3.3
4.96	4.78	-3.6
average	4.86	2.1
st. deviation	0.08	1.6
%CV	1.59	
1.01	1.09	7.6
1.01	0.93	-7.8
1.01	0.99	-2.4
average	1.00	5.9
st. deviation	0.08	3.1
%CV	7.86	
1.01	1.15	13
1.01	0.95	-5.5
1.01	0.92	-8.8
1.01	0.95	-6.1
1.01	0.96	-5.4
average	0.98	7.8
st. deviation	0.09	3.4
%CV	9.23	
0.52	0.46	-12
0.52	0.49	-5.8
0.52	0.52	-0.8
0.52	0.46	-12
0.52	0.37	-29
average	0.46	12
st. deviation	0.06	11
%CV	12.04	

Analysis by: Operator II

Apparatus: WT1

The results in Table VI indicate that the WT method for determination of F in blood does not suffer from significant carryover as can be judged from system blank values measured after 25.42 and 101.36 ppm samples. A slight increase in values is observed; however, we do not believe that the differences are significant enough to impact our results since the method is intended to be used primarily for samples containing less than 25 ppm of fluorine.

The accuracy of the method was evaluated across the concentration range from 0.17 to 101.36 ppm as indicated by results in Table V and VI. These data should be correlated with the reproducibility data shown in Table VII and VIII. As expected, the relative error values increase with decreasing F concentration, reaching 27% at 0.55 ppm level.

The reproducibility of the method at approximately 25 ppm was evaluated by two different operators using two different WT's. The results from these experiments are summarized in Table VII. It can be seen that at these concentration levels both operators achieved approximately the same reproducibility (%CV's of 3.86 and 4.11 %) as well as accuracy (relative errors of 4.3 ± 3.7 % and 3.4 ± 2.5 %). The reproducibility evaluation at concentrations of 5 ppm and lower are shown in Table VIII. Each data set in this table was analyzed by the same operator (Operator I) on different days. As expected, these results indicate that both precision (%CV's) and accuracy (relative errors) improve with increasing concentration. Although this operator did not conduct the reproducibility experiments at high concentrations (25 ppm) using the same apparatus as for the low concentrations, the results in Table VIII and VII indicate that he should still be able to achieve acceptable results using the current apparatus at higher than 5 ppm concentrations.

It is interesting to note that this operator achieved worse reproducibility and accuracy at 25.60 ppm using WT2 (Table VII) than he achieved at 4.96 ppm using WT1 (Table VIII). This may be due to the fact that the 4.96 ppm samples were analyzed at a later time and the operator had the opportunity to gain more experience with WT combustion method in general. This would suggest that the operator technique while operating WT apparatus is one of the important factors influencing the accuracy and reproducibility of the method. This is also supported by the fact that the other operator was not able to achieve accurate and reproducible results at concentrations of 5 ppm and lower using both WT's by the time the data above were generated. Therefore, a conclusion can be made that at low concentration levels these validation data should be linked to a specific operator using a specific WT apparatus.

Operator I was however, able to generate quantitatively valid data using WT2 at a later time. These data are summarized in Tables IX - XIII.

Data in Table VIII and IX indicate that Operator I/WT2 and Operator II/WT1 achieved equivalent precision and accuracy at the concentration level of approximately 5 ppm F in blood. This is indicated by similar values of standard deviations, %CV's (precision), and % relative errors (accuracy) of their respective replicate measurements at this concentration level.

Table IX.

Reproducibility evaluation of WT/F-ISE determination of F in whole rat blood at 5 ppm^a

Actual F concentration (ppm)	Determined F concentration (ppm)	relative error (%)
5.30	4.99 ^b	-5.8
5.02	4.85	-3.4
5.02	4.84	-3.5
5.02	5.07	1.0
5.02	4.94	-1.5
5.02	4.91	-2.1
average	4.92	2.9
st. deviation	0.09	1.7
%CV	1.88	

Analysis by: Operator I

Apparatus: WT2

^a samples separated by lines were replicate analyses of the same sample within a particular day

^b value not used for average, standard deviation, and %CV calculation because of different target F concentration.

Table X.

Evaluation of background fluorine levels in blank rat blood by WT/F-ISE^a

Actual F concentration (ppm)	Determined F concentration (ppm)
blank blood	0.08
blank blood	0.10
blank blood	0.37
blank blood	0.10
blank blood	0.21
blank blood	0.31
blank blood	0.34
blank blood	0.35
blank blood	0.29
blank blood	0.24
blank blood	0.26
blank blood	0.26
blank blood	0.15
average	0.24
st. deviation	0.099
%CV	42.19

Analysis by: Operator I

Apparatus: WT2

^a samples separated by lines were replicate analyses of the same sample within a particular day

Table XI.

Reproducibility evaluation of WT/F-ISE determination of F in whole rat blood at 1 ppm^a

Actual F concentration (ppm)	Determined F concentration (ppm)
1.04	1.03
1.04	1.06
1.02	1.20
1.02	1.20
1.00	1.16
1.00	1.19
1.00	1.19
1.00	1.06
1.00	1.09
1.00	1.09
1.04	1.19
1.04	1.24
1.02	1.16
1.02	1.29
1.01	1.19
1.01	1.20
1.01	1.26
1.01	1.22
1.01	1.28
1.01	1.22
1.01	1.16
1.01	1.22
1.12	1.16
1.12	1.23
1.12	1.26
1.12	1.13
1.12	1.10
average	1.18
st. deviation	0.069
%CV	5.9

Analysis by: Operator I
Apparatus: WT2

^a samples separated by lines were replicate analyses of the same sample within a particular day

Table XII.

Reproducibility evaluation of WT/F-ISE determination of F in whole rat blood at 0.5 ppm^a

Actual F concentration (ppm)	Determined F concentration (ppm)
0.50	0.62
0.50	0.70
0.55	0.79
0.55	0.65
0.52	0.70
0.52	0.64
0.51	0.70
0.51	0.79
0.51	0.67
average	0.70
st. deviation	0.061
%CV	8.7

Analysis by: Operator I

Apparatus: WT2

^a samples separated by lines were replicate analyses of the same sample within a particular day

Careful examination of the data presented in Table XI and XII shows that the results in both cases are about 0.2 ppm higher than the targeted concentration of F spiked into the samples. The constant nature of this systematic error suggests that the blank blood of these samples already contained some background level of F. If this is true and the operator performed the analysis in the consistent manner, standard deviations of these measurements should be ideally the same. Indeed, the values of standard deviations of the replicate measurements for these levels are equivalent. In order to evaluate the background F level for these samples, blank blood samples were analyzed using the same blood that was used to prepare the samples in Table XII and most of XI. The results are shown in Table X. As seen from the results shown in this table, the blank blood contained on average about 0.245 ppm F. The reproducibility of this measurement is not as great as for the other results shown, because these results are very close to the method limit of detection (LOD, see later for LOD, LOQ determination). If the average F blank concentration is above LOD of the method, the value can be deemed to be due to background F and it can be subtracted from the results shown in Table XI and XII.

The results of F blood analyses at 1 and 0.5 ppm (Table XI and XII) corrected for background F are shown in Table XIII.

Table XIII.

Total F blood analyses at 1 and 0.5 ppm levels (from Table XI, XII) corrected for background F.

Average actual concentration (ppm)	Average measured concentration (ppm)	Standard deviation (ppm)	F content corrected for background (ppm)	% relative error
blank (n = 13)	0.24	0.099		
1.03 (n = 27)	1.18	0.069	0.94	-8.9
0.52 (n = 9)	0.70	0.061	0.46	-11.3

Analysis by: Operator I

Apparatus: WT2

Table XIII shows that after the correction of the applicable results for the background F in blank blood, the results show acceptable accuracy. This correction was possible only because the average background F concentration in blank rat blood was at the LOD of the method with the Operator I/WT2 (see Table XIV for LOD data). The determined blank blood concentration can therefore be deemed to be due to F and can be subtracted. It should be mentioned however, that the stated value (see Table X) is only an estimate because the value was under the LOQ (Table XIV). Hence, the great variability in this determination. It is realistic to expect some background level of F in blank rat blood because of the F composition of their diet. The F-ISE method described in this report was used to evaluate the concentration of ionizable inorganic F⁻ in the animal facility water that is generally fed to the rats in the Haskell Laboratory. The water contained 0.9 ppm F⁻. In addition, the standard rodent diet also contains F in the range of 8-15 ppm (data supplied by Purina Mills, diet supplier). Because of the great variation of the F content in this diet (almost 100% variability) not all the blood will contain the same background F concentration. We had no indication that the blood used for data generated by Operator II, and Operator I at 5 ppm level contained any detectable background F levels. The blood used by Operator I/WT2 for blank, 0.5 and 1 ppm levels came from the same batch of animals.

Determination of limit of detection (LOD) and limit of quantitation (LOQ) of determination of total F in blood by WT/F-ISE.

Assuming that the results produced by WT/F-ISE behave according to normal distribution, the LOD and LOQ can be estimated from the standard deviation of replicate measurements of concentration levels close to the LOQ (not to exceed 10x LOQ). LOD was estimated as 3x the average standard deviation at low F concentration levels.

Limit of quantitation (LOQ) of WT/F-ISE method for analysis of total fluorine in blood was established taking into account the accuracy needed for the majority of

upcoming toxicological studies for which this method is intended. LOQ was estimated as 6x the average standard deviation at low F concentration levels. LOD and LOQ data for total F determination in blood by WT/F-ISE by both operators are shown in Table XIV.

Table XIV.

LOD and LOQ of determination of total F in blood by WT/F-ISE.

	Average actual concentration (ppm)	Average standard deviation of F determination (ppm)
Operator I/WT2	blank (n = 13)	0.099
	0.52 (n = 9)	0.061
	1.03 (n = 27)	0.069
	5.02 (n = 5)	0.093
	average	0.081
	LOD (3x avg. st. dev.)	0.24
	LOQ (6x avg. st. dev.)	0.49
Operator II/WT1	0.52 (n = 5)	0.055
	1.01 (n = 8)	0.085
	4.96 (n = 5)	0.077
	average	0.072
	LOD (3x avg. st. dev.)	0.22
	LOQ (6x avg. st. dev.)	0.43

Data shown in Table XIV indicate that both operators achieved equivalent LOD and LOQ with their respective WT's. Therefore, LOD of the WT/F-ISE determination of total F in whole blood is 0.24 ppm with both operator/WT combinations. Any results lower than this value will be reported in the future studies as "ND", standing for "not detected". LOQ of the method is 0.5 ppm and any data lower than 0.5 ppm but higher than or equal to 0.24 ppm will be reported as "< 0.5" indicating that F presence was detected but its concentration was too low to quantitate it with the required degree of accuracy.

The ability of automated F-ISE measurement of F⁻ in WT samples to produce accurate data is critical for the success of WT/F-ISE method. As mentioned earlier (part A, sect. 1.2) performance of F-ISE should be evaluated daily based by the deviation of calibration curve slope from the theoretical slope value. Since the F-ISE is operated at the limit of its performance as specified by manufacturer and within the non-linear portion of its response, the slope value agreement does not guarantee the accuracy of the results. As a result, measurement of a Quality Control (QC) sample was introduced whenever the F-ISE measurement was used. The QC sample was prepared independently by a third party. It was based on aqueous NaF. The targeted F⁻ concentration in the QC sample was 1 ppm. This sample was treated the same way as samples from WT (sample size 0.5g used,

NaAc and F⁻ spike added and filled to 150g). Since the QC sample was not processed through WT, its calculation cannot be based on WT system blank due to its different matrix. A 'QC system blank' was prepared instead (sample size 0.0000g used, NaAc and F⁻ spike added and filled to 150g) and used exclusively for QC calculation. A result from QC analysis based on 'QC system blank' should match the QC targeted concentration within 15 % as indicated by the results shown in Table IV. If this criterion is not fulfilled, a corrective action should be taken to ensure the accuracy of F-ISE data. Introduction of QC to the procedure impacts the sample throughput only slightly because all the F-ISE measurement is automated. However, higher confidence in the data can be obtained and it can also be used as a great troubleshooting aid when the results are not as expected.

Conclusions

Validation of determination of total fluorine in blood by Wickbold Torch in combination with direct potentiometric fluoride detection with Fluoride Ion Selective Electrode has been performed. It has been found that F-ISE determination of fluoride in samples processed through WT is operator-independent. Best possible accuracy and precision can be achieved if the F-ISE is not exposed to water and buffered 10 ppb F⁻ solution is used for the electrode rinse. In addition, calibration standards should be measured from highest to lowest concentration, and volumetric glassware should be used whenever possible for volume transfers. The electrode should not be exposed to high F⁻ concentrations as well and the electrode performance check and storage conditions outlined in its manufacturer manual should not be followed because they are intended for its operation in the linear portion of the electrode response. To ensure the accuracy of F-ISE measurement a QC sample was measured and the validity of its result compared to the targeted concentration value was assessed daily. The electrode performance was evaluated each time it was used based on the deviation of the calibration curve slope from the theoretical slope value. Short-term electrode storage should be done in a 20 ppb reference solution.

WT combustion method was evaluated for blood samples and it was found that the method does not suffer from significant carryover. Reproducibility and accuracy at high concentration levels was evaluated with different operators with different WT's. The results were generally equivalent. However, at low concentration levels, it was observed that the results greatly depend on operator/WT apparatus combination, which suggests the influence of the operator technique on the accuracy and precision of the method. For this reason, a careful consideration should be given if the data presented in this report were applied to any measurements requiring adjustments in the operator technique. This include (but is not limited to) analysis of different matrices and the operators using different apparatus than they used to generate the validation data in this report. That is, this validation report is valid only for total fluorine determination in blood and similar matrix (such as plasma, red blood cells) and for the particular operators using their respective WT's by which they generated these validation data. In addition, for validity of any data of future studies, it is assumed that the operators perform the analyses in exactly the same manner as they did when they generated data presented in this report. When more data becomes available for different matrices and different operator/WT combinations, attachments to this report will be written.