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Findings of an External Quality Assessment Scheme for Determining Aluminum in Dialysis Fluids and Water

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The analytical performance of laboratories participating in the dialysis fluids and water aluminum program of the Guildford External Quality Assessment Scheme over the period 1986–1993 has been reviewed. For aluminum concentrations >3.33 μmol/L in dialysis fluids, the between-laboratory CV has fallen from ~36% to 23%, whereas for specimens of water the reduction was from 36% to ~18%. Improvements at lower concentrations were less impressive. Performance of individual participants varied; only a few consistently provided accurate results. Many of the participants are able to measure serum aluminum well, so lack of expertise is not responsible for poor results. We suggest that matrix effects associated with different specimen types have a significant influence on performance and that due account is not always taken of these factors. Resolution of these problems would be aided by appropriate reference materials.

Indexing Terms: *interlaboratory performance/reference materials*

Patients with chronic renal failure requiring treatment by hemodialysis or continuous ambulatory peritoneal dialysis are at risk of developing aluminum toxicity. To protect these patients, it is essential that they be included in a program of regular surveillance. In addition to careful clinical observations the program should include measurement of aluminum in serum, dialysis fluids, other treatment solutions, and water (*1*). Guidelines concerning the frequency of measurements and the concentrations indicative of failure to provide protection have been elaborated by responsible organizations (*1, 2*); if monitoring programs are to be effective, however, the analytical procedures adopted for measuring aluminum in these specimens must be accurate and precise.

The Robens Institute organizes a large international project to assess the quality of measurements of trace elements in biological fluids, including aluminum in serum, dialysis fluids, and water (*3*). Results from this external quality assessment (EQA) scheme show that many laboratories now measure aluminum in serum with good accuracy and precision. This was not initially the situation (*4, 5*), but performance has gradually improved over recent years (*6–8*): The proportion of participants whose performance scores fell within the range deemed to be satisfactory were 18%, 23%, and 35% in 1986, 1988, and 1990, respectively. In the UK, where various initiatives have been undertaken to improve performance, this figure (in 1993) was >80%.

Progress in the measurement of aluminum in dialysis fluids and water has not been presented, but previous investigations of performance demonstrated a generally unsatisfactory situation. In the most-recent reports (1988), the between-laboratory CVs were two- to threefold greater than for analyses of serum specimens having equivalent concentrations of aluminum (*6, 7, 9*). Here we present further assessments to determine whether changes in performance have occurred in parallel with those observed for the measurement of aluminum in serum.

Materials and Methods

Organization of the EQA scheme. Each month, two dialysis fluid specimens and two water specimens are prepared and sent to laboratories participating in the EQA scheme. Participants measure the aluminum concentrations and report their results to the organizers within 3 weeks of the date of dispatch. Summary reports are prepared from these results and posted to participants to allow them to compare their own values with the consensus data.

Preparation of dialysis fluid and water specimens. Dialysis fluid concentrate (Renalyte; Macarthays Medical Ltd., Romford, UK) 28.5 mL, is pipetted into a 1-L volumetric flask containing ~900 mL of aluminum-free reverse-osmosis-purified (Elga, High Wycombe, UK) water (RO-water). Nitric acid, 10 mL, and aluminum nitrate (Al, 3.7 mmol/L) solution (in an appropriate volume to increase the final concentration of aluminum by a predetermined amount) are added, made to volume (1 L) with RO-water, and thoroughly mixed.

For water analysis, ~900 mL of tap water or RO-water is placed in a 1-L volumetric flask. Nitric acid and aluminum nitrate solution are added as for the dialysis fluid, made to 1 L with RO-water, and mixed.

These solutions are dispensed into labeled, plastic, aluminum-free 10-mL tubes (Teklab, Sacriston, UK) for distribution to participants of the Trace Elements EQA Scheme. On some occasions (see below), specimens were sent in 25-mL plastic Universal containers (Bibby Sterilin, Stone, UK), which are consistently found to be free from contamination with aluminum.

All glassware and other items are cleansed before use by soaking overnight in 100 mL/L HCl and repeatedly rinsed in RO-water to ensure that traces of aluminum are removed.

To determine whether the large variation of results, in comparison with the serum aluminum EQA program, was a consequence of unsatisfactory specimens distrib-

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uted for analysis, we undertook a series of experiments at the early stages of the scheme to investigate specimen container, stability, and the effect of nitric acid preservative. The statistical significance of differences between results was determined by the paired *t*-test.

Reports. The monthly report summarizes results for the four specimens previously distributed to participants. After exclusion of outliers (results >3 SD from the mean of all results given for a specimen), the mean, SD, and CV of the refined data are determined. These calculations are printed onto a report form together with a note of the amount of aluminum added during preparation and a histogram display of the distribution of results for each sample.

Further calculations. The procedure used to assess performance of individual laboratories in the serum aluminum program of the EQA scheme was applied to the dialysis fluid and water data (*3*). Proximity to the mean $(x - \bar{x})$ was calculated for each result reported by a participant, and this value was plotted onto the zonal chart (Fig. 1). The range described by the boundary of the inner zone is ~±75% at very low concentrations, decreasing to ±7.5% at the highest concentration. At the completion of the period under review, the number of points within the inner and outer target zones was determined as a percentage of the results reported. For performance to be deemed acceptable, 60% and 80% of the points should fall within the inner and outer zones, respectively, or the combined percentages should be at least 140%.

Results

Results from the experiments to monitor the quality of the specimens are shown in Tables 1 and 2. Dialysis fluid specimens kept in either 10- or 25-mL plastic containers gave similar means and SDs, indicating no measurable contamination from the tubes (Table 1); however, aluminum could be lost from dilute solutions. Recovery of added aluminum was low (55.5–79.4%) if the samples were not acidified; when nitric acid was added (final concentration of 10 mL/L), no aluminum was lost (Table 1). Possible long-term changes were investigated for as long as 8 months. Aluminum concentrations of 0.93–3.70 μmol/L in dialysis fluids and water remained essentially unchanged throughout the study;

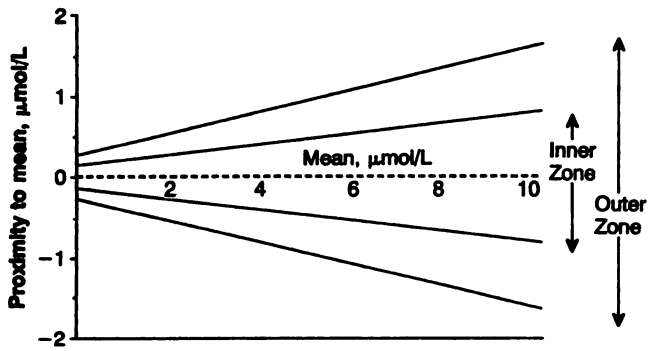

Fig. 1. Chart showing the inner and outer target zones on which the proximity of a result to the consensus mean $(x - \bar{x})$ can be plotted.

Table 1. Effect of container-type and sample acidification on aluminum measured in dialysis fluid specimens.

	Al, μmol/L	
Container size and type	Added	Mean ± SD measured
No acidification		
10-mL, plastic	0.93	17.55 ± 9.55
10-mL, plastic	1.85	27.76 ± 12.91
25-mL, Universal	0.93	19.86 ± 8.80
25-mL, Universal	1.85	32.99 ± 13.70
Nitric acid, 10 mL/L, added		
10-mL, plastic	0.93	29.42 ± 9.32[a]
10-mL, plastic	1.85	52.70 ± 17.72[b]
25-mL, Universal	0.93	29.27 ± 8.52[b]
25-mL, Universal	1.85	51.35 ± 12.12[a]

[a,b] Significantly different from nonacidified samples: [a] $P < 0.01$, [b] $P < 0.001$. Differences between 10- and 25-mL containers were not significant.

the spread of results was also unaltered, as indicated by the values for SDs (Table 2).

The between-laboratory CVs for 7 successive years (up to March 1993) are shown in Table 3. Because precision is affected by analyte concentration in the sample, especially at concentrations close to the limit of detection of the analytical method, we present the values in four concentration bands. Between-laboratory CVs represent only a crude indication of performance by the entire group of participants; nonetheless, these data show that there has been some overall improvement, most evident at concentrations >1.11 μmol/L. As the between-laboratory CVs have improved, a difference has emerged between the analysis of water and dialysis fluids, with slightly better results being obtained for the simpler (water) matrix.

Despite the wide range of results reported for each specimen by the participants, the mean values were very close to the anticipated concentrations. Analytical recovery of aluminum added to dialysis fluids was consistently very close to 100% (mean 101.8%, range 92.7–123.9% for added aluminum at 0.37–3.70 μmol/L). Results for aluminum added to water specimens (0.37–11.11 μmol/L) were slightly less: 99.0% (90.3–110.8%). Fig. 2 shows these correlations for the 48 specimens distributed during 1992–93.

The individual performances of three laboratories who have regularly participated in the scheme since its inception are shown in Fig. 3. These three laboratories were selected to show individual performance because they had consistently reported good results in the serum aluminum EQA program. The three participants illustrate quite different trends in performance according to type of specimen analyzed. Laboratory 1 achieved a good standard of performance throughout most of the period under review. Results for water are not quite as good as for dialysis fluids but met the criteria for acceptable performance, and a slow trend towards lower scores was reversed in the 1992–93 period. The two other laboratories both experienced periods when performance deteriorated dramatically. Laboratory 2 was subject to prob-

Table 2. Stability of aluminum concentrations (mean ± SD) measured in specimens of dialysis fluids and water stored at −20°C.

Months stored	Dialysis fluid + Al, μmol/L				Water + Al, μmol/L			
	0.74	1.11	2.22	2.96	0.93	1.11	2.22	3.70
0	0.90 ± 0.29	1.37 ± 0.43	2.63 ± 0.76	3.04 ± 0.58	0.96 ± 0.26	1.34 ± 0.33	2.36 ± 0.65	3.36 ± 0.56
1	—	1.40 ± 0.44	2.52 ± 0.55	—	—	1.23 ± 0.43	2.24 ± 0.57	—
3	0.96 ± 0.36	1.40 ± 0.39	2.45 ± 0.71	—	1.09 ± 0.34	1.29 ± 0.39	2.21 ± 0.55	—
4	—	—	—	3.07 ± 0.51	—	—	—	3.47 ± 1.02
8	0.89 ± 0.30	—	—	2.97 ± 0.62	1.02 ± 0.28	—	—	3.59 ± 0.92

Differences between stored and unstored specimens were not significant.

Table 3. Mean between-laboratory CVs (%) for measurement of aluminum in dialysis fluid and water at different concentrations, 1986–1993.

Year	N[a]	Al in dialysis fluid, μmol/L				Al in water, μmol/L			
		0–1.11	1.12–2.22	2.23–3.33	>3.33	0–1.11	1.12–2.22	2.23–3.33	>3.33
1986–87	30	92.3 (2)[b]	—	33.3 (2)	36.3 (6)	88.1 (2)	27.5 (1)	97.6 (1)	36.4 (6)
1987–88	40	74.5 (2)	23.5 (2)	35.6 (2)	30.3 (4)	128.6 (1)	29.4 (3)	30.2 (2)	23.5 (3)
1988–89	44	59.9 (5)	38.7 (3)	—	—	71.5 (2)	50.1 (1)	—	—
1989–90	52	45.1 (1)	31.5 (2)	29.8 (4)	29.5 (2)	52.8 (2)	32.3 (4)	27.3 (3)	25.7 (1)
1990–91	55	36.0 (5)	27.0 (5)	21.9 (6)	—	35.2 (5)	24.8 (5)	23.1 (2)	23.9 (3)
1991–92	50	55.3 (3)	26.8 (4)	25.9 (6)	26.3 (5)	81.9 (1)	25.5 (3)	21.9 (2)	17.8 (10)
1992–93	56	79.2 (5)	29.2 (9)	23.4 (6)	23.2 (4)	71.2 (5)	22.7 (3)	19.7 (2)	18.4 (14)

[a] No. of laboratories regularly reporting within each period; the number of results reported for individual samples varied a little from month to month.
[b] % CV (and no. of samples within each concentration group).

lems in 1989–90 that were more apparent for dialysis fluids than for water; since then, the measurement of aluminum in water has been acceptable, but analysis of dialysis fluids has not been as successful. Laboratory 3 showed improvements in performance until 1990–91, when measurements with both dialysis fluids and water deteriorated; the assay for dialysis fluid quickly recovered but problems with water are still evident.

During 1992–93, the number of participants who returned results for at least 5 of the 12 distributions were 58 for dialysis fluids and 59 for water. Of these, 21 (36%) reported results for dialysis fluids indicative of acceptable performance according to the criteria defined above. For measurement of aluminum in water, 30 (51%) gave acceptable results.

Discussion

This lack of agreement, indicative of poor performance at many laboratories, makes it impossible to effectively put into practice the guidelines of the European Economic Community Resolution (*1*). The reasons for this widespread unsatisfactory analytical performance are not known but probably relate to factors such as contamination, components of the samples that interfere with the analytical procedures, and the absence of stable reference materials (*5, 7, 9*). The experiments carried out to examine the quality of the EQA specimens, together with our own checks during the preparation of these samples, indicate that the material distributed to participants was satisfactory.

It is unlikely that contamination is a general problem. Many of the participants obtain acceptable results

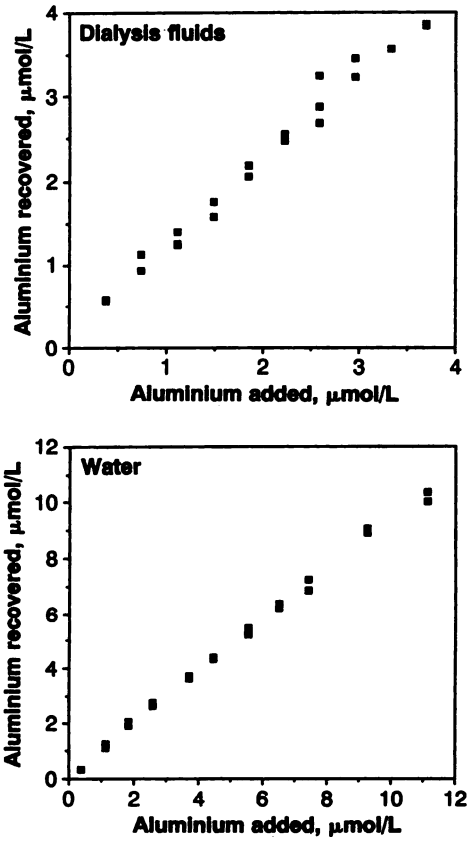

Fig. 2. Recovery of aluminum added to dialysis fluid specimens (*top*) or water specimens (*bottom*) during the 1992–93 cycle of the EQA scheme.

The consensus means calculated from results of all participants (after exclusion of outliers) are plotted vs the added aluminum.

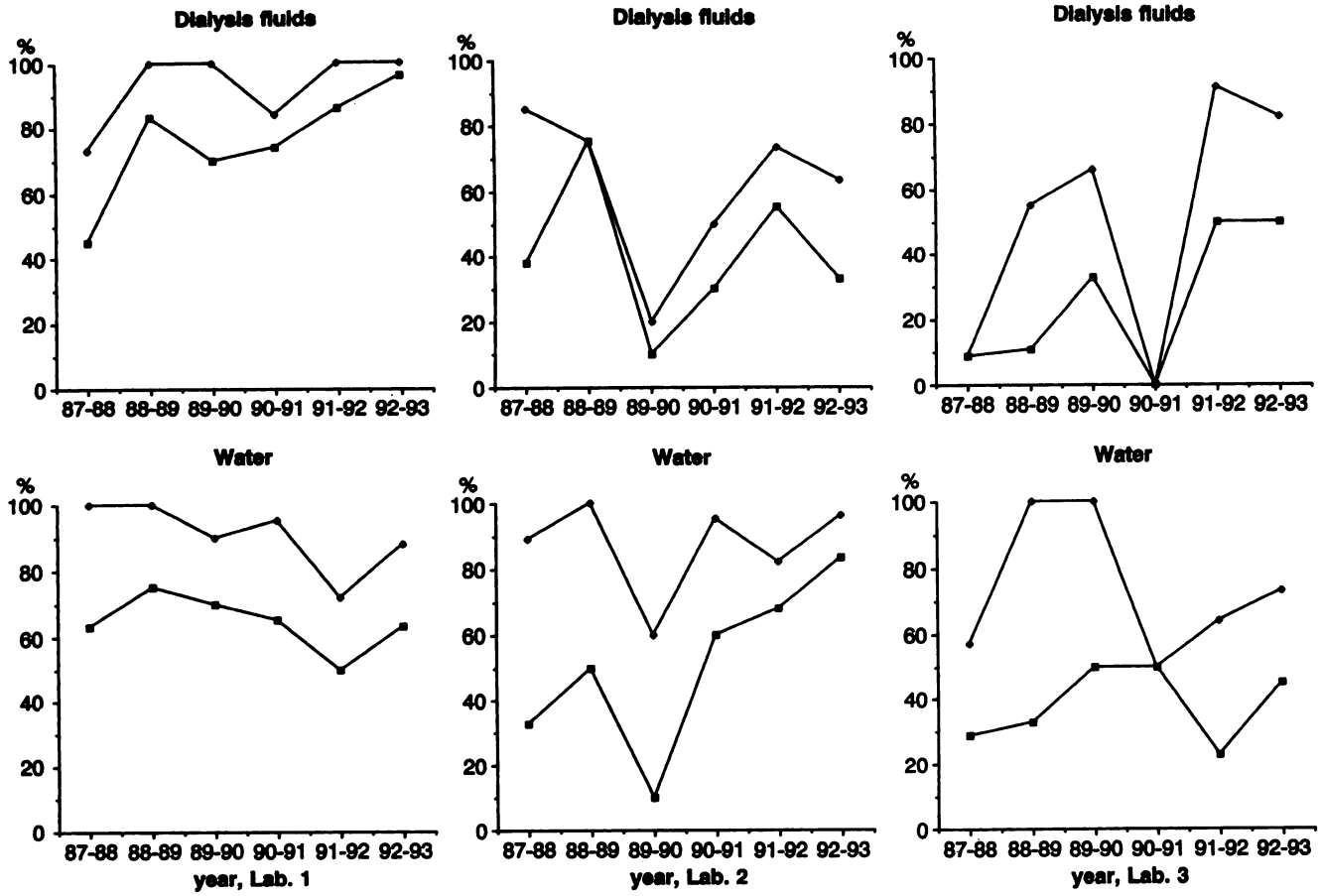

Fig. 3. Performance for three laboratories throughout 1987 to 1993.
Performance is shown for each annual cycle as the percentage of results within the inner (⊡) and outer (◈) target zones as plotted on chart in Fig. 1.

for measurement of aluminum, indicating that they have established working environments where contamination has been more or less eliminated. The recovery rates of 100% on a regular basis (Table 1 and *Results*) suggest that the specimens do not become contaminated. In addition, the consistent satisfactory performance of participants such as Laboratory 1 (Fig. 3) would not be observed if there were contamination problems with the specimens.

Table 3 and Fig. 3 demonstrate differences in performance for specimens of dialysis fluids and water. Further differences between analyses of these fluids and of serum samples have also been noted (*6*). Our laboratory had showed that a single method could be used to measure aluminum in these specimens (*10*); a few years later, however, we found that this was no longer possible, and the procedure had to be modified to give specific conditions for use with dialysis fluids and others for use with water. Although we do not have an explanation for the change in performance, we presume it involves interactions between the sample matrix and small but significant changes in the analytical instruments (e.g., quality of graphite used, temperatures actually attained within the furnace, rates of temperature increase). Such changes would influence the optimum graphite furnace settings, the calibration procedures, and other analytical variables. Similar changes in instrumental performance have probably occurred in other laboratories (at least one participant communicated the same experience to us) but not all will necessarily have appropriately adjusted their methodologies.

The improvement in performance observed for measurements of aluminum in serum has been stimulated by the provision of a series of reference materials that laboratories can use for internal quality control (*5, 6*). This has become particularly evident in the UK, where enhanced scrutiny of performance in the last 2 years has shown that rigorous use of these materials was a key factor in the achievement of good performance (*6–8*). The availability of similar materials for dialysis fluids and water is crucial to further development of reliable analytical methods and their regular use in laboratories responsible for monitoring aluminum exposure of patients. However, there are significant problems with the manufacture of reference materials for these specimens and none are readily available. Containers made of different materials (e.g., glass, polythene, polypropylene) and obtained from several suppliers have been tested for contamination with use of serum and 10 mL/L nitric acid as trial solutions. With glass containers the aluminum concentration of the contents was increased by as much as 0.93 μmol/L (serum) or 8.15 μmol/L (10 mL/L nitric acid) when mixed for 2 h. Contamination was less marked with plasticware but was seen in some contain-

ers. Furthermore, the amounts leached were not consistent, either within or between batches of tubes (*11, 12*). Aluminum can also be lost from dilute solution by adsorption onto the surface of storage containers (*13, 14*). Although acidification will stabilize the aluminum in solution, this can, in turn, exacerbate any contamination from containers, thus giving rise to erratic results with some unrealistically high values (*11, 12*). For many biological samples long-term stability is achieved by lyophilization, which also makes for easier storage conditions; however, freeze-drying vials are invariably manufactured from glass to provide the strength necessary to maintain a vacuum and are likely to be a source of contamination. Also, it is not certain whether material is lost from very dilute solution during the removal of water.

Despite these difficulties it is imperative that some attempt be made to prepare reference materials that can be used to monitor the accuracy of methods for measuring aluminum in dialysis fluids and water. Unfortunately, patients are still at risk from aluminum toxicity (*15*), but inadequate analytical procedures should not be responsible for allowing toxicity to occur.

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