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# Quality Control of Busulfan Plasma Quantitation, Modeling and Dosing: An Interlaboratory Proficiency Testing Program

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**ABSTRACT**

Background:

Personalizing busulfan doses to target a narrow plasma exposure has improved the efficacy and lowered the toxicity of busulfan-based conditioning regimens used in hematopoietic cell transplant (HCT). Regional regulations guide interlaboratory proficiency testing for busulfan concentration quantification and monitoring. To date, there have been no comparisons of the busulfan pharmacokinetic modeling and dose recommendation protocols used in these laboratories. Here, in collaboration with the Dutch Association for Quality Assessment in Therapeutic Drug Monitoring and Clinical Toxicology, a novel interlaboratory proficiency program for the quantitation, pharmacokinetic modeling, and dosing of busulfan in plasma

was designed. The methods and results of the first two rounds of this proficiency testing are described herein.

Methods: A novel method was developed to stabilize busulfan in N,N-dimethylacetamide, which allowed shipping of the proficiency samples without dry ice. In each round, participating laboratories reported their results for two proficiency samples (one low and one high busulfan concentrations) and a theoretical case assessing their pharmacokinetic modeling and dose recommendations. All participants were blinded to the answers; descriptive statistics were used to evaluate their overall performance. The guidelines suggested that answers within ±15% for busulfan concentrations and ±10% for busulfan plasma exposure and dose recommendation were to be considered accurate.

Results: Of the four proficiency samples evaluated, between 67% and 85% of the busulfan quantitation results were accurate (i.e., within 85-115% of the reference value). The majority (88% round #1; 71% round #2) of the dose recommendation answers were correct.

Conclusion: A proficiency testing program by which laboratories are alerted to inaccuracies in their quantitation, pharmacokinetic modeling, and dose recommendations for busulfan in HCT recipients was developed. This round of proficiency testing suggests that additional educational efforts and proficiency rounds are needed to ensure appropriate busulfan dosing.

Keywords: busulfan, proficiency testing, therapeutic drug monitoring, pharmacokinetics, quality control



## BACKGROUND

High-dose busulfan is frequently used in allogeneic hematopoietic cell transplantation (HCT) conditioning regimens. Typical HCT busulfan doses range from 2–4 mg/kg/day for 1–4 days, resulting in a total dose of 3.2–16 mg/kg.[1] The busulfan area under the plasma

concentration-time curve (AUC) value has been associated with important post-transplant outcomes in different conditioning regimens[2-5], with higher rates of graft rejection[6-8] or relapse[9] being closely associated with low busulfan AUC values. High busulfan AUC values (over treatment) have been linked to higher rates of hepatotoxicity[6,10-14] and non-relapse mortality.[13] In busulfan followed by cyclophosphamide ~~(BU/CY)~~ conditioning, personalized busulfan regimens developed using patient-specific busulfan clearance rates, often referred to as busulfan therapeutic drug monitoring (TDM), has been linked to reduced hepatotoxicity rates (from 75% to 18%[15]) and reduced graft rejection (from 26% to 4%[16]). Since busulfan is administered over a short period of time (i.e., 1–4 days), busulfan TDM is time-sensitive, which forces most HCT centers to evaluate their busulfan samples using local laboratories.

Recently, the American Society for Blood and Marrow Transplant's (now the American Society for Cellular Therapy and Transplantation (ASTCT)) Committee on Practice Guidelines sought to produce an evidence-based guideline for personalizing busulfan-based conditioning.[4] Unfortunately, they could not update or create new target AUCs because the published data is too heterogeneous and lacks adequately powered and sufficiently controlled studies.[4] To overcome this challenge, we invited numerous busulfan TDM laboratories to discuss solutions to resolve these evidence gaps.[4] From the identified concerns, two projects were prioritized: 1) busulfan plasma exposure unit harmonization[17] and 2) busulfan quantitation, pharmacokinetic modeling, and dose recommendations (BuQMD), which is reported here.

At present, evaluating busulfan quantitation is overseen by regional and national regulators. To the best of our knowledge, there have been no interinstitutional comparisons of busulfan pharmacokinetic modeling and dose recommendations completed to date. The busulfan proficiency testing program described here includes assessing each task involved in busulfan TDM, including: 1) the quantitation of busulfan plasma concentrations; 2) the

pharmacokinetic modeling of these concentration-time points; and 3) busulfan dose recommendations.

The aim of this program was to minimize the risk of busulfan dosing errors and facilitate the production of multicenter databases to evaluate the relationships between busulfan AUC and HCT outcomes. Here, we report on the program's development and the results of the first two rounds of proficiency testing.

## MATERIALS AND METHODS

This BuQMD project was developed as an external proficiency testing program and designed to facilitate the validation of busulfan TDM result accuracy. The Drug Analysis and Toxicology division (KKGT) of the Dutch Foundation for Quality Assessment in Medical Laboratories (SKML, www.kkgt.nl)[18] has existing infrastructure designed to facilitate proficiency testing for drug quantitation and dosage recommendations.

### Participating Laboratories

The co-authors of this paper extended invitations to various laboratories within the HCT scientific community to participate in the BuQMD proficiency testing program. These invitations were sent electronically and snowballing strategies (where respondents could nominate or extend an invitation to other relevant stakeholders) were used to identify participating laboratories.

### Proficiency Test Kit Development

We aimed to develop an affordable method for sending busulfan proficiency samples to international sites while maintaining their stability. Aqueous busulfan solutions exhibit temperature-dependent stability, and since busulfan solutions degrade more rapidly at higher temperatures[19], samples are typically shipped on dry ice. However, international dry-ice shipping is cost-prohibitive. To address these technical challenges, we developed a method to

stabilize busulfan in plasma samples so that we could produce a test kit that could be shipped on ice packs and eliminate the need for dry ice.

Each proficiency test kit was prepared using a multi-step process. Each kit contained two busulfan proficiency samples (one low and one high busulfan concentration sample). First, a research staff member (Arjen Punt) from Utrecht University Medical Center would produce the two proficiency samples from a 1000 mg/L busulfan stock solution prepared using N,N-dimethylacetamide. This stock solution was stored in 1 mL aliquots at -80 °C and shown to be stable (recovery within 95%) for 4.5 years. For proficiency round #1 testing, the stock solution was diluted to either 3.2 mg/L (low concentration) or 28 mg/L (high concentration) in N,N-dimethylacetamide. For proficiency round #2, the stock solution was diluted to 5 mg/L (low concentration) or 16 mg/L (high concentration) in N,N-dimethylacetamide. Then, 90 □L of each sample was diluted in 1 mL of blank calf serum to produce sample kits with the following concentrations: 0.264 mg/L (low) and 2.312 mg/L (high) (proficiency round #1) and 0.413 mg/L (low) and 1.321 mg/L (high) (proficiency round #2). These theoretical concentrations were used as the reference values when evaluating the participating laboratory's quantitation of these samples. To assess the accuracy of the sample kit dilutions, we went on to quantify the proficiency samples using liquid chromatography-mass spectrometry (LC-MS) in a European co-operation for Accreditation ~~(EA)~~ ISO15189-accredited laboratory (see Supplementary Method 1,http://links.lww.com/TDM/A472). These values were shown to be within 15% (i.e., 85-115%) of the theoretical value and, therefore, considered acceptable. Subsequently, the proficiency samples were sent to KKGT for distribution, with the time between preparation and shipping to KKGT not exceeding 3 weeks.

These proficiency samples were stored for 1 week in a -80 °C freezer before shipping as part of the proficiency test kits to the participating laboratories. These kits included: 1)

busulfan samples in N,N-dimethylacetamide; 2) polypropylene micro tubes (1.5 mL, Brand, Wertheim, Germany) with blank plasma; 3) instructions for preparation of the busulfan proficiency samples; and 4) an internal temperature sensor to monitor the kit's contents. The recipient laboratories evaluated their temperature recordings on receipt but did not report them to KKGT.

**Assessment of Busulfan Quantitation (Q of BuQMD)**

For each proficiency round, the participating laboratories were asked to quantitate the proficiency samples. All participating laboratories were blinded to the concentrations of these samples, and their results were considered accurate if they fell within 15% of the reference value. This 15% was found to be consistent with the guidelines described by the Food and Drug Administration and the European Medicines Association for bioanalytical validation.[20,21]

**Assessment of Pharmacokinetic Modeling and Dose Recommendations (MD of BuQMD)**

We developed a set of theoretical clinical cases to assess the participating laboratories' busulfan pharmacokinetic modeling and dose recommendations. These cases were revised iteratively. After the cases were completed, four co-authors independently (i.e., blinded to each other's answers) answered the questions. These four answers were averaged, and this average was used as the reference value.

The participating laboratories were asked to answer the questions in one case per proficiency round. Proficiency round #1's case had four questions and proficiency round #2's case had two questions (Supplementary Method 2,http://links.lww.com/TDM/A472). In proficiency round #1, the three most common methods for calculating busulfan plasma exposure (AUC in mg□h/L ), AUC in □Mol□min ($\frac{micromole}{liter} \times minute$) or concentration at

steady state (Css) in ng/ml ($\frac{nanogram}{milliLiter}$ – were accepted as units of measurement.[17] After we published our results that AUC expressed as mg□h/L was the preferred harmonized busulfan plasma exposure unit,[17] proficiency round #2 only included this unit. Answering these questions was optional. Each question was open-ended and each answer was entered as free-text and not restricted to a specific numerical range. These cases were answered in a blinded manner ensuring that no laboratories knew each other's answers.

For each question, answers within ±10% of the reference value were defined as accurate. This 10% reference value was chosen because busulfan exposure can be targeted to a single exposure value[22] or a narrow range (e.g. a Css of 800–900 ng/ml[23], which equates to a daily AUC of 19.2–21.6 mg×h/L using the harmonized busulfan plasma exposure unit[17]).

**Data Analysis**

R Studio (Version 1.3.1073) and R (version 4.0.2) were used for all data analysis, with all the descriptive statistical evaluations described in the results. The results of each participating laboratory were anonymized in accordance with the relevant KKGT privacy policies.

**RESULTS**

To date, two rounds of busulfan proficiency testing have been completed (Figure 1). Proficiency testing round #1 was completed between May 31, 2019, and September 22, 2019, and involved 27 laboratories while proficiency testing round #2 was completed between December 11, 2019, and January 21, 2020, and involved 25 laboratories. Most of the participating laboratories used LC-MS to quantitate their busulfan samples (Table 1), but any method was allowed.

**Quantitation of Plasma Busulfan Concentrations (Q of BuQMD)**

In proficiency round #1, one laboratory (3.7%) appeared to have a typographical error, with its values being 1000 times higher than any of the other laboratories, while in proficiency round #2, one laboratory (4%) reported a value 1000 times higher than the other laboratories. While their original answers were recorded in the analyses, they are not included in Figure 1.

In proficiency round #1, 18 out of 27 laboratories (67%) reported a busulfan value within 15% of the reference value for the low concentration samples while 23 out of the 27 laboratories (85%) where within 15% of the reference value for the high concentration samples. For proficiency round #2, 18 out of 25 laboratories (72%) reported a value for the low concentration samples within 15% of the reference value while 17 out of these 25 laboratories (68%) reported a concentration within 15% of the reference value for the high concentration samples.

**Pharmacokinetic Modeling and Dose Recommendations (MD of BuQMD)**

The reference values and answers used in both rounds of proficiency testing are described in Table 2. The participating laboratories were not required to answer the pharmacokinetic modeling and dose recommendation questions.

Proficiency round #1 included three questions related to pharmacokinetic modeling, requiring laboratories to estimate the plasma busulfan exposure in the three most commonly used units of measurement and then to make a dose recommendation based on these values. In proficiency round #1, 15 (55%) and 16 (59%) out of the 27 laboratories answered the plasma exposure and dose questions, respectively. Two of these laboratories (11.7%) appeared to have made a typographical error in their answers with one laboratory answering an AUC of mg×h/L of 24,279 for question one and another answering a Css of 0.98 ng/mL for question three. Their original answers were retained in the analysis. While their original

answers were used in the analysis, the typographic errors were ‘adjusted’ to assumed answers in Supplementary Figure 1,http://links.lww.com/TDM/A471. Supplementary Figure 1,http://links.lww.com/TDM/A471 shows the distribution of the answers and indicates the reference value for each question. The proportion of laboratories supplying accurate answers to the questions regarding busulfan plasma exposure ranged from 75% (Css in ng/mL) to 88% (AUC in µMolxmin) (Table 2), with the majority (88%) of the dose recommendations shown to be accurate.

Proficiency round #2 had two questions: to use pharmacokinetic modeling to estimate the plasma busulfan exposure using the harmonized unit of mg×h/L and another regarding the dose recommendation based on this value. In proficiency round #2, 17 out of the 25 (68%) laboratories completed these questions. A total of two laboratories (11.7%) appeared to have made a mistake in their answer to question one with the total AUC in mg×h/L. Specifically, one laboratory answering an AUC of mg×h/L of 72.8 and another laboratory answered an AUC of mg×h/L of 110. Two of these laboratories (11.7%) appeared to have made a typographical error in their answers with one laboratory answering an AUC of mg×h/L of 24,279 for question one and another answering a Css of 0.98 ng/mL for question three.

however, their original answers were retained in the analysis. While their original answers were used in the analysis, the typographic errors were ‘adjusted’ to assumed answers in Supplementary Figure 2,http://links.lww.com/TDM/A471. The majority (82%) of the pharmacokinetic modeling results, AUC (mg×h/L), and the majority (71%) of the dose recommendation answers were accurate (Table 2).

## DISCUSSION

Our main findings from the two rounds of busulfan proficiency testing completed to date were as follows: 1) the majority of laboratories could quantify the busulfan concentrations using our sample kits with results within 15% of the reference value; 2) between 75% and 88% of the calculated busulfan plasma exposure values were within 10% of the reference value; and 3) 88% (round #1) and 71% (round #2) of the laboratories recommended a busulfan dose within 10% of the reference value. This proficiency testing program provides a framework for comparing busulfan quantitation, modeling, and dose recommendations between laboratories used by HCT centers for busulfan TDM. If continued, this program can help to assure the accuracy of busulfan TDM for both patient care and research.

Many drugs that undergo TDM, including busulfan[4], do not have high-quality evidence for their target exposure or plasma concentration.[24] The ASTCT Clinical Practice Guideline Committee could not establish these target AUCs because the published data is too heterogeneous and lacks adequately powered and sufficiently controlled studies.[4] Proficiency testing could facilitate more rigorous, multicenter studies evaluating the association between busulfan TDM and clinical outcome. The inclusion of busulfan exposure data from the Center for International Blood and Marrow Transplant Research (CIBMTR) database is likely expand this even further. Collecting this type of data is important, especially as a survey suggested that 50% to 60% of HCT centers who report to CIBMTR used busulfan TDM.[25] Such studies could facilitate the development of evidence-based guidelines for target busulfan AUC values in different HCT conditioning regimens designed for specific disease settings. The association between busulfan AUC and outcome may differ based on the HCT conditioning regimen, the patient's age, and their underlying disease.[4] Ideally, these studies would help to improve overall survival by maximizing efficacy and minimizing toxicity.

Target busulfan AUC values can be refined using future studies designed to produce sufficient power and using appropriate controls.

This proficiency testing program overcame the rapid degradation of busulfan in aqueous solutions at higher temperatures[19], by resuspending samples in N,N-dimethylacetamide, reducing the need to transport these samples on dry ice. The first two rounds of proficiency testing used two busulfan reference samples, which meets the ISO criteria[26] but may not meet regional regulatory standards for between-laboratory comparisons.

To our knowledge, this is the first proficiency testing program evaluating busulfan pharmacokinetic modeling and dose recommendations. The accuracy of the participants' answers, defined as those answers falling within 10% of the reference value, for the pharmacokinetic modeling and dose recommendation evaluations were disappointing. For the pharmacokinetic modeling task, 12% to 25% of the busulfan exposure estimation answers were inaccurate (Table 2). For the busulfan dose recommendation, 12% or 29% were inaccurate (Table 2). Proficiency round #1 had two outliers that were possibly typographical errors, but these types of errors can be difficult to recognize if an HCT center typically uses a different busulfan plasma exposure unit. For example, one answer in proficiency round #1 included a Css value of 0.98 ng/mL, which might be difficult to recognize as an error if the HCT center typically uses mg□h/L, where an AUC range of 4.8.–5.4 mg×h/L after one dose of IV busulfan administered every 6 hours is possible. This data suggests that there is an urgent need for more educational and regulatory efforts to improve the accuracy of their busulfan pharmacokinetic modeling and dose recommendations. Asynchronous online certifications in busulfan TDM may be beneficial.

In the absence of a community standard, we decided to define values within 10% of the reference value as accurate for the pharmacokinetic modeling and dose recommendations. This margin was based on our experience and knowledge of the currently available evidence;

however, this margin may need to be addressed in the future. In addition, our proficiency sample testing procedures have three limitations. First, errors could have been made in the preparation of the kits. Second, we relied on the participating laboratories to assess the temperature readings following shipment. Finally, we allowed an extended time period between sample receipt and busulfan quantitation reporting. Thus, some proficiency samples may have been stored in unknown conditions for an extended period. It is unknown how sample loss/degradation contributes to the inaccuracy of these results. Future proficiency testing will need to collect data around the temperature of the test kits during shipping and the storage conditions before quantitation. We may also consider reducing the timeframe for evaluation to prevent storage induced variation.

Our results suggest that additional work within the busulfan dose personalization for HCT is needed to improve accuracy. Education, certification, and mandatory participation in busulfan proficiency testing, in partnership with various organizations, such as the KKGT with the ASTCT, and the European Blood and Marrow Transplant (EBMT) groups – may be valuable tools in improving these outcomes and harmonizing busulfan TDM.

## CONCLUSION

In conclusion, we have described the results of two rounds of busulfan proficiency testing using a novel method for maintaining busulfan stability when shipped on cold packs. While most participating laboratories were shown to be fairly accurate in each individual task (busulfan concentration quantitation, pharmacokinetic modeling, and dose recommendations) there is room for improvement. Certification of busulfan TDM proficiency and mandated participation in busulfan proficiency testing for each of the tasks in busulfan TDM may improve the accuracy of busulfan dose personalization and potentially improve both patient and research outcomes.

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**Figure Legends**

Figure 1. Busulfan quantitation results for proficiency round #1 (Fig 1A) and #2 (Fig 1B). The x-axis represents the participating laboratories (lab) and the y-axis represents the percentage of the reference value. The black dots represent the low busulfan concentration sample and the white triangles represent the high busulfan concentration sample. The dashed line represents the 85$^{th}$ percentile and the dotted line represents the 115$^{th}$ percentile. Outliers > 250 mg/L removed.

Table 1. Analytical methods used to quantitate busulfan concentrations by self-report of participating laboratories

| Analytical method | Proficiency round #1[a] | Proficiency round #2[a] |
|---|---|---|
| Immunoassay | 0 | 0 |
| Gas chromatography–mass spectrometry | 4 (15%) | 4 (16%) |
| High-performance liquid chromatography | 1 (4%) | 1 (4%) |
| Liquid chromatography–mass spectrometry | 22 (81%) | 20 (80%) |
| Total number of participating laboratories | 27 | 25 |
| [a]Shown as number (%) | | |

Table 2. Answers to the pharmacokinetic modeling and dose recommendation questions provided by the participating laboratories.

| Proficiency round | Questions (Qu) | Reference value | Answer median (range) | % of accurate[a] answers |
|---|---|---|---|---|
| #1 | Qu 1. What is the AUC with Dose 1 in mg h/L? | 24.0 | 24.1 mgxh/L (18.6–24,279)[b] | 80% (12 of 15)[b] |
| | Qu 2. What is the AUC with Dose 1 in µmol/min? | 5847 | 5868 µmol/min (4518–6631) | 88% (14 of 16) |
| | Qu 3. The concentration at steady state (Css) in ng/mL is another commonly used descriptor in busulfan measurements. If the dosing frequency is measured over a 24-hour period (every 1440 minutes), what is the Css for a dose of 1 ng/mL? | 993 | 1007 ng/mL (0.98–1133)[c] | 75% (12 of 16)[c] |
| | Qu 4. Based on your calculated dose 1 exposure (AUC and/or Css), what dose (in mg) would you recommend for dose 2 in order to achieve the desired targeted exposure over the course of the busulfan conditioning? | 260 | 267 mg (240–1080) | 88% (14 of 16) |
| #2 | Qu 1. What is the AUC (mg h/L) for day 1 with dose 1? | 15.2 | 15.1 mgxh/L (10.2–110) | 82% (13 of 17) |
| | Qu 2. Based on your calculated dose for 1 AUC, what dose (in mg) do you recommend for dose 2 in order to achieve the desired targeted exposure over the course of busulfan conditioning? | 285 | 290 mg (167–895) | 71% (12 of 17) |

[a] within 10% of the reference value; [b] if the answer of 24, 279 mg h/L is assumed to be a typographical error with the intended answer being 24.279 mg h/L, then the range is (18.6–27.2) and 87% (13 of 15) of the answers are accurate. [c] If the answer of 0.98 ng/mL is assumed to be a typographical error with the intended answer being 980 ng/mL, then the range is (750–1133) and 81% (13 of 16) of the answers are accurate.

Figure 1. Busulfan quantitation results for proficiency round #1 (Fig 1A) and #2 (Fig 1B). The x-axis represents the participating laboratories (lab) and the y-axis represents the percentage of the reference value. The black dots represent the low busulfan concentration sample and the white triangles represent the high busulfan concentration sample. The dashed line represents the 85$^{th}$ percentile and the dotted line represents the 115$^{th}$ percentile. Outliers > 250 mg/L removed.