REF BSF-RGT

MyCare™ Oncology Busulfan Assay Kit

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IVD	*in vitro* Diagnostic Device	LOT	Batch Code
	Consult Instructions for Use		Manufacturer
	Temperature Limitation		Use By Date
REF	Catalog Number	EC REP	Authorized Representative in the European Community
R1	Reagent 1	R2	Reagent 2
	(N) x Gently invert reagents (R1 and R2) *N* number of times prior to use		

Indications For Use

The MyCare Oncology Busulfan Assay Kit is intended for the in vitro quantitative measurement of busulfan in human heparinized plasma using automated clinical chemistry analysers. Measurements obtained can be used to aid in management of individuals prescribed intravenous busulfan.

Summary and Explanation of the Test

Busulfan (1,4-butanediol dimethanesulfonate; Busulfex®) is a bifunctional alkylating agent indicated for use in combination with cyclophosphamide (CY) as a conditioning regimen prior to allogeneic hematopoietic progenitor cell transplantation (HPCT) for chronic myelogenous leukemia (CML).[1] Busulfan is also used for myeloablation before hematopoetic stem cell transplantation for other malignant diseases such as acute myeloid leukemia, myelodysplastic syndromes, acute lymphatic leukemia and non-malignant diseases like metabolic syndromes, haemoglobinopathy, and immunodeficiency, among others.[2]

For myeloablation before transplant, busulfan is frequently administered as a two hour infusion every six hours over four days for a total of 16 doses. Therapeutic drug monitoring for busulfan dose adjustment is recommended in the Busulfex package insert for the first cycle of treatment.[1] The therapeutic target is the area under the curve (AUC) of 56 – 86 mgxh/L (900-1350 µM min) for pediatric patients.[1] To calculate the AUC, blood samples are taken at the end of infusion, four hours after the start of infusion and before the next dose (trough). Therapeutic Drug Monitoring (TDM) of busulfan should be considered to minimize sinusoidal obstruction syndrome, lower graft rejection rates, and reduce relapse rates.[3]

The MyCare Oncology Busulfan Assay Kit (US Patent No. 7,893,220) is a homogeneous two-reagent nanoparticle agglutination immunoassay used for detection of busulfan in human plasma. It is based upon the principle of measuring changes in scattered light or absorbance which result when nanoparticles aggregate. This aggregation is measured at wavelengths between 400 and 650 nm using automated clinical chemistry analysers. Multivalent drug-conjugates serve as a binding partner to antibodies selective for busulfan which are covalently attached to the surface of nanoparticles. In the absence of free busulfan, this reaction creates large aggregates, resulting in a solution that scatters incident light and leads to an increase in the observed absorption of the solution. When a sample containing busulfan is introduced, the agglutination reaction is partially inhibited. Antibody bound to sample drug is no longer available to promote nanoparticle aggregation, resulting in less scattering of incident light and lower observed absorption of the solution. Thus, a classic inhibition curve with respect to busulfan concentration is obtained with the maximum absorption occurring with low levels of drug and minimum absorption occurring with high levels of drug. Monitoring the changes in scattered light or absorbance as a function of drug levels results in a concentration-dependent curve.[4-5]

Reagents

The kit contains sufficient reagent for 100 tests.*

MyCare Oncology Busulfan Assay Kit **REF** BSF-RGT	Quantity x Volume
Reagent 1 **R1** Reaction buffer that contains drug-conjugate and protein in a buffered solution	1 x 9.5 mL
Reagent 2 **R2** Nanoparticle reagent that contains monoclonal antibody bound to nanoparticles in a buffered solution	1 x 9.5 mL

*Analyser dependent

Warnings and Precautions

- For In Vitro Diagnostic Use Only.
- Exercise the normal precautions required for handling all laboratory reagents.
- Follow reagent handling instructions. Improper mixing of reagents can affect assay performance.
- Materials of human origin were tested for HIV1, HIV2, Hepatitis B and Hepatitis C by FDA-approved methods and the findings were negative. However, as no test method can rule out the potential risk of infection with absolute certainty, the material must be handled just as carefully as a patient sample. In the event of exposure, the directives of the responsible health authorities should be followed.
- All components of the busulfan assay contain less than 0.1% sodium azide. Avoid contact with skin and mucous membranes. Flush affected areas with copious amounts of water. Seek immediate medical attention if reagents are ingested or come into contact with eyes. When disposing of such reagents, always flush with large amounts of water to prevent accumulation of azide.

Reagent Handling

The busulfan assay reagents are ready to use.

Before use, mix the reagents by gently inverting five times, avoiding the formation of bubbles.

Mix the reagents before pouring them into any analyser-specific (secondary) reagent carriers. Before placing analyser -specific (secondary) reagent carriers on the analyser, mix the reagents by gently inverting five times, avoiding formation of bubbles.

Storage and Stability

Store reagents refrigerated at 2-8°C. Do not freeze.

When stored and handled as directed, unopened reagents are stable until the expiration date on the label. Improper storage of reagents can affect assay performance.

Sample Collection and Handling

Sodium heparinized plasma is required.

Collect samples directly after the end of infusion, at 4 hours after the start of infusion, and directly before the next infusion.

Busulfan is unstable. Keep whole blood samples in a wet ice slurry or refrigerated at 2 – 8°C. Centrifuge whole blood and process to plasma within two hours of collection. Plasma may be kept refrigerated at 2 – 8°C for up to 24 hours before analysis.

For longer storage of plasma, freeze the sample at -80°C for up to 12 months and at -20°C for up to 3 months. Do not freeze whole blood samples.

Ensure the plasma sample is thawed and thoroughly mixed before measuring.

Procedure

Assay

To run the assay, see the instrument specific application sheet and appropriate analyser operator’s manual.

Instruments

Reagents may need to be transferred to analyser-specific reagent containers (see Reagent Handling).

Materials Provided:

REF BSF-RGT – MyCare Oncology Busulfan Assay Kit

Materials Required – Provided Separately

REF BSF-CAL – MyCare Oncology Busulfan Calibrator Kit

REF BSF-CON – MyCare Oncology Busulfan Control Kit

Specimen Dilution Procedure

Samples containing greater than 2,000 ng/mL busulfan can be diluted automatically or manually 1:2 (1 part sample plus 2 parts water) to give an upper range of 6,000 ng/mL.

Calibration

To perform a calibration, see the instrument specific application sheet and appropriate analyser operator's manual.

Perform a full calibration using the six calibrators from the Busulfan Calibrator Kit. Verify the calibration by testing the low, medium, and high controls from the Busulfan Control Kit.

Calibration Frequency

Calibration is recommended:

- After reagent kit lot change,
- After performance of monthly instrument maintenance,
- As required following quality control procedures.

Quality Control

Each laboratory should establish its own QC procedures for the busulfan assay. All quality control requirements and testing should be performed in accordance with local, state, and/or federal regulations or accreditation requirements. Good laboratory practice suggests that at least two concentrations of quality control be tested each day patient samples are assayed, and each time a calibration is performed. Reassess control targets and ranges following a change of reagent (kit) or control lot.

Results

The results of the MyCare Oncology Busulfan Assay Kit are used to calculate an AUC or C_{ss} (concentration at steady state).

$$Css = \frac{\text{AUC}}{dosing\ frequency}$$

Results are reported in ng/mL. The conversion factor for µM is 0.0041 x ng/mL = 1 µmol/L.

Limitations of the Procedure

As with all analyte determinations, the MyCare Oncology Busulfan Assay Kit should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

The busulfan assay has been validated for sodium heparin plasma. Do not use plasma separator tubes.

Do not use proficiency or external quality control samples containing organic solvents.

As with any assay utilizing mouse antibodies, the possibility exists for interference by human anti-mouse antibodies (HAMA) in the sample. Samples containing such antibodies can potentially produce erroneous busulfan results, which are inconsistent with the patient's clinical profile.

Citalopram at concentrations of 5500, 3700 and 1900 ng/mL tested with 325 ng/mL of busulfan elevated the busulfan result 48%, 29%, and 17% respectively. High therapeutic concentrations of citalopram may cause a bias in the results.

Expected Values

TDM of busulfan is used to personalize dose to a target exposure as area under the plasma concentration-time curve (AUC) or concentration at steady state. Busulfan concentrations are used to calculate busulfan exposure as AUC.[3] Busulfan drug concentrations should not be the only means of therapeutic drug management. The assay should be used in conjunction with information available from clinical evaluations and other diagnostic procedures.

AUC can be calculated using a variety of methods; such as non-compartmental analysis using the trapezoidal rule and pharmacokinetic (PK) modeling.[2]

Specific Performance Data

Typical performance data for the busulfan assay are shown below. Results obtained in individual laboratories may differ from these data.

Precision

Within-laboratory precision and repeatability were verified throughout the measuring range according to CLSI Guideline EP05-A3.[6] Three Busulfan Control Kit controls and four normal human plasma pools spiked with busulfan (Spike 1, 2, 3, 4) were tested. The samples were assayed twice a day for twenty days using three lots of reagents and two analysers.

The following is representative data from one lot of reagents run on one analyser.

Sample Type		Assigned Value (ng/mL)	N	Mean (ng/mL)	Repeatability	Within-laboratory
					%CV	%CV
Controls	Low	225	80	250	4.6%	6.1%
	Medium	450	80	461	3.1%	3.9%
	High	900	80	910	1.8%	2.8%
Plasma	Spike 1	325	80	328	4.7%	5.7%
	Spike 2	600	80	615	4.0%	4.8%
	Spike 3	1100	80	1124	2.1%	2.9%
	Spike 4	1500	80	1531	2.6%	3.1%

Limit of Quantitation (LoQ) and Limit of Detection (LoD)

The lower limits of quantitation and detection were established using CLSI guideline EP17-A2.[7]

LoQ

The LoQ was determined with an accuracy goal at the LoQ of ≤ 35% total error (Westgard model). The LoQ of the busulfan assay is 187 ng/mL.

LoD

The LoD is the lowest amount of analyte that can be reliably detected (≥ 95% of results greater than the limit of blank.). The LoD of the busulfan assay is 96 ng/mL.

Measurement range

The measurement range of the busulfan assay is 187 – 2,000 ng/mL.

Specificity

Metabolism

Busulfan is predominately metabolized by conjugation with glutathione, both spontaneously and by glutathione S-transferase (GST) catalysis. This conjugate undergoes extensive oxidative metabolism in the liver.[1] Metabolites reported in plasma and urine include tetrahydrothiophene (THT), THT-1-oxide, sulfolane, and 3-hydroxy-sulfolane.[8,9]

Specificity for the following metabolites and cross-reactants was tested in the absence and presence of 325 and 1500 ng/mL busulfan.

Compound	Tested at (ng/mL)	% Bias
THT	100	2%
THT-1-oxide	500	3%
Sulfolane	800	3%
3-hydroxysulfolane	500	3%

No Significant bias was observed in samples with the following endogenous interferents at the given levels.

Interferent	Level	
Rheumatoid Factor	508 IU/mL	
Human Serum Albumin	10.7 g/dL	107 g/L
Human Immunoglobulin G	11.7 g/dL	117 g/L
Human anti-mouse antibodies	100 ng/mL	
Icteric Interference	44 mg/dL	752 µmol/L

Interferent	Level	
Lipemic Interference	711 mg/dL	8 mmol/L
Hemolysate	1,025 mg/dL	
Uric acid	1.5 mg/dL	89 mmol/L

Cross-reactivity

The following compounds did not interfere with the busulfan assay: the assay bias was <23%.

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
Acetaminophen	200,000	Acetylsalicylic acid	500,000
Acyclovir	66,000	Albuterol	1,000
Alendronate sodium	1,000	Allopurinol	60,000
Alpha - tocopherol	129,300	Alprazolam	2,000
Amantadine	10,000	Amikacin sulfate	144,000
Amisulpride	1200	Amitriptyline	1,000
Amlodipine besylate	100	Amoxicillin	80,000
S (+)-amphetamine	1,000	Azathioprine	2,600
Baclofen	3,000	Benztropine	600
Biotin	3,600	Bupropion	3,000
Buspirone	20	Caffeine	108,000
Calcium carbonate	315,000	Carbamazepine	45,000
Cefalexin	200,000	Ceftriaxone	84,000
Celecoxib	10,000	Cetirizine dihydrochloride	4,400
Chlordiazepoxide	6,900	8-chlorotheophylline	3,000
Chlorpromazine HCl	3,300	Cimetidine	30,000
Ciprofloxacin	12,000	Clindamycin	51,000
Clofarabine	13,200	Clonazepam	300
Clotrimazole	2,400	Codeine	2,000
Cortisol	300	(-)-cotinine	2,000
Cyclophosphamide	549,000	Cyclosporine	1,800
Deferasirox	75,000	Desloratadine	600
Dextromethorphan	1,000	Diazepam	30,000
Diphenhydramine HCl	6,000	Docosahexaenoic acid ethyl ester	150,000
Doxycycline HCl	35,000	Duloxetine	200
Erythromycin	138,000	Estradiol	1.2
Ethanol	6,000,000	Etoposide	42,000
Fentanyl	600	Fluconazole	25,500
Fludarabine	5,200	Fluoxetine HCl	1,000
Flurazepam	500	Fluticasone propionate	10
Folic acid	15	Gemcitabine	16,000
Gentamycin sulfate	30,000	Ibuprofen	500,000

Compound	Tested at (ng/mL)	Compound	Tested at (ng/mL)
Indinavir sulfate	400	Itraconazole	6,000
Kanamycin	90,000	Lamivudine	10,500
L-ascorbic acid	60,000	Levetiracetam	180,000
Lidocaine	15,000	Lorazepam	1,000
Meclizine	500	Melphalan	4,500
Methotrexate	1,360,000	Methylprednisolone	7,900
Metronidazole	123,000	Morphine	7,800
Naproxen sodium	500,000	Nicotine	1,000
Nicotinic acid	54,000	Nordiazepam	5,000
Omeprazole	8,400	Ondansetron	350
Oxazepam	5,000	Oxycodone	500
Pantothenic acid	1,800	Penicillin G	30,000
Penicillin V	42,000	Phenobarbital	690,000
Phenytoin	60,000	Posaconazole	2,100
Potassium EDTA	1,000	Prednisolone	3,000
Pregabalin	22,500	Procainamide	48,000
Prochlorperazine	3,500	Promethazine	1,200
R,R (-)-pseudoephedrine	10,000	S,S (+)-pseudoephedrine	10,000
Pyridoxine HCl	100	Quinidine	15,000
Ranitidine	10,500	Retinol	4,000
Riboflavin	200	Rifampicin	48,000
Salicylic acid	500,000	Sodium fluoride	900
Sodium Heparin	50 U/mL	Streptomycin Sulfate	258,000
Sulfamethoxazole	400,000	Temazepam	5,000
Thiamine HCl	500	Thiotepa	30,000
Tobramycin	33,000	Topiramate	30,000
Trazodone HCl	10,000	Triazolam	40
Trimethoprim	42,000	Valproic acid	500,000
Vancomycin	120,000	Vitamin B12	1
Vitamin D2	1,200	Vitamin K1	10
Voriconazole	18,000	Vorinostat	2,800
Warfarin	75,000	Zolpidem hemitartrate	5,000
Zonisamide	120,000	Zopiclone	400

Recovery

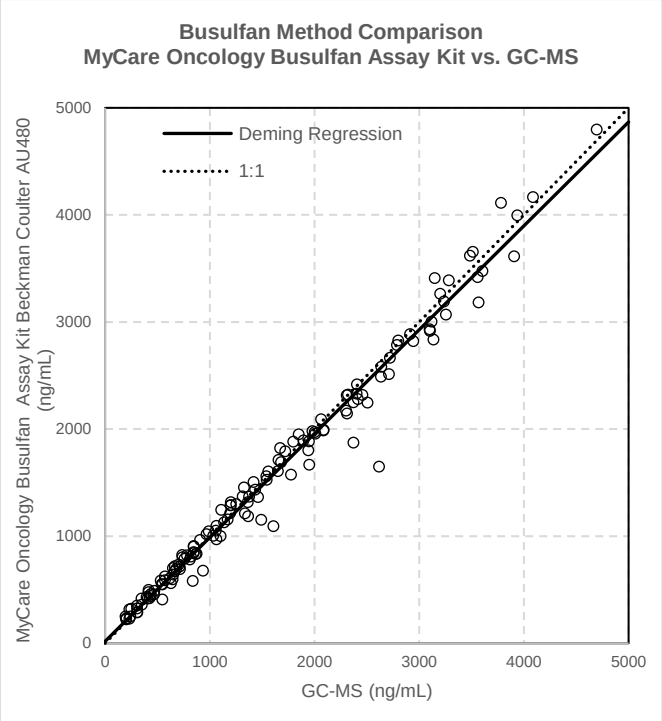
The recovery of busulfan was assessed in the 3 controls, and clinical pools measured for the EP05-A3 precision performance study. The percent recovery was determined by dividing the mean measured concentration of each sample by the expected concentration of busulfan. The mean recovery deviation ranged from -1% to 4%.

Linearity

The linearity of the busulfan assay was verified according to CLSI guideline EP6-A.[6] Eleven linearity samples covering the measuring range were prepared in human plasma spiked with busulfan. Linear regression gave a slope of 1.000 (CI 95%: 0.988 – 1.013) and an intercept of 29 (CI 95%: 14 - 45) with an R = 0.999. Deviation from linearity (n=5) was -12%. The assay was linear across the measuring range from 187 to 2000 ng/mL.

Method Comparison

Results of the busulfan assay were compared to a validated GC-MS, using samples from patients taking busulfan according to CLSI guideline EP09c.[10] Deming regression analysis was performed with 208 busulfan patient samples. Results are shown for one lot.



Regression Statistics Busulfan Assay Kit vs. GC-MS	
Slope	0.97
Intercept	18
Correlation Coefficient (R)	0.9917
N	208
Concentration Range (GC-MS)	171 – 4,696

Package Insert References:

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4. McNally AJ, Goc-Szkutnicka K, Li Z, Pilcher I, Polakowski S, Salamone SJ. An online immunoassay for LSD: comparison with GC-MS and the Abuscreen RIA. *Journal of analytical toxicology.* 1996;20(6):404-408.
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