

UHAS-MIDA Software Package: Mass Isotopomer Distribution Analysis-Applying the Inverse Matrix for the Determination of Stable Isotopes of Chromium Distribution during Red Blood Cell Labelling

Daniel A. Abaye^{1*}, Emmanuel B. Odoom², Ernest Y. Boateng¹, Irene A. Agbo¹, John-Bosco Diekuu², Samuel Agana²

¹Department of Basic Sciences, School of Basic and Biomedical Sciences, University of Health and Allied Sciences, Ho, Volta Region, Ghana

²ICT Directorate, University of Health and Allied Sciences, Ho, Volta Region, Ghana
Email: *dabaye@uhas.edu.gh

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Abstract

Clinical assessment of fluid volume status in children during malaria can be taxing and often inaccurate. During malaria, changes in fluid volume are rather multifarious and estimating this parameter, especially in sick children is very challenging for clinicians who frequently rely on indices such as long capillary refill times, tachycardia, central venous pressure and decreased urine volume as guides. Here, we present the *UHAS-MIDA*, an open-source software tool that calculates the red blood cell (RBC) concentration and blood volume during malaria in children determined using a stable isotope of chromium (⁵³Cr as the label) by gas chromatography-mass spectrometry in selective ion monitoring (GC/MS-SIM) analysis. A key component involves the determination of the compositions of the most abundant naturally occurring isotopes of Cr (⁵⁰Cr, ⁵²Cr, ⁵³Cr), and converting the proportions into a 3 × 3 matrix. To estimate unknown proportions of chromium isotopic mixtures from the measured abundances of three ions, an inverse matrix was calculated. The inverse together with several inputs is then used to calculate the corrected MS ion abundances. Thus, we constructed the software tool *UHAS-MIDA* using HTML, CSS/Bootstrap, JavaScript, and PHP scripting languages. The tool enables the user to efficiently determine RBC concentration and fluid volume. The source code, binary packages and associated materials for UHAS-MIDA are freely available at

https://github.com/bentil078/Abaye-et-al_UHASmida.

Keywords

Mass Isotopomer Distribution Analysis, Inverse Matrix, GC/MS, Chromium Stable Isotopes, Blood Volume, Red Blood Cell Mass

1. Introduction

Central to all cellular processes in living organisms is metabolism which underpins life itself. When a compound, such as a nutrient, an antigen or a drug is introduced into the human body, among other responses, enzymes are activated to act upon these compounds. The metabolites are actively transported through pathways and they can be converted to other appropriate metabolites, e.g. for synthesis, into energy molecules, modulated, and or eliminated. Only a few quantitative methods really allow for direct determination of metabolic fluxes in living systems, and one of which is based on *in vivo* experiments using relatively simple stable isotope-labeled precursors, such as ^{13}C -glucose [1], ^2H - and ^{13}C -ascorbic acid [2] [3], ^{13}C -amino acid [4] [5], ^{13}C - and ^{15}N -amino acid [6], $^2\text{H}_2\text{O}$ [7] and ^{53}Cr [8]. The transfer of the label to the metabolic network and the specific isotope distribution in the metabolic products can then be taken as confirmation of metabolic pathways and fluxes during the experimental period. An appropriate set of technological tools is then required to quantitatively determine the abundances of the isotopes, especially when several metabolites are involved.

In a simple setting such as the growth of bacteria in a controlled environment with a single carbon or nitrogen source, it is possible to develop accurate metabolic flux distributions from the data [9] [10] [11]. The data are typically generated using gas chromatography-mass spectrometry (GC/MS) or liquid chromatography-mass spectrometry (LC/MS). The data output is composed of, for example, the mass-to-charge ratio (m/z) values of compounds and fragments of molecules, the number of atoms in a compound, the elemental composition of compounds, and the relative MS response (intensity). The output is then compared with the unlabeled compounds under the same experimental conditions. The computational analysis of the isotopomers, especially when data are from GC/MS results typically contains derivatives (e.g. silylated) and therefore, requires numerical manipulation [12] [13].

In humans, labeling techniques are much more complex and require more judicious care and ethical issues. During malaria in children, fluid volume management is crucial for optimum supportive care and depends on knowledge of changes in fluid balance and red cell mass that occur in the disease [8]. The direct determination of fluid volume loss using stable isotopes, deuterated water ($^2\text{H}_2\text{O}$) [7], red blood cell loss using ^{53}Cr [8] [14] [15] and for ^{18}F tracing cardiac metabolism [16] in disease have been reported.

2. Background

Several computational analytical tools, generally referred to as mass isotopomer

distribution analysis (MIDA), that is, calculations involving the enrichment of isotopomeric groups, have been developed and used (e.g [3] [12] [17] [18]). Generally, the developed algorithm treats experimental raw data generated from MS; the mass spectrometric response or intensities of metabolic products (either ^{13}C , ^{15}N or ^{53}Cr) are analysed on the basis of their m/z values and the number of C, N or Cr atoms, respectively, in the given molecule, derivative or fragment. For example, Zeeshan *et al.* [17] [18], calculated the overall ^{13}C - or ^{15}N -enrichment, and the relative and molar contribution of isotopomers using Braumann's least squares algorithm [19]. Bluck *et al.* [3], however, developed a 3×3 matrix and its inverse matrix to achieve the mass isotopomer distribution of ^2H - and ^{13}C -ascorbic metabolism in adults, and this approach was applied to determine ^{53}Cr -labeled red blood cell loss during malaria in children from Gabon [8]. The key steps in using ^{53}Cr as a label for determining red blood cell loss are described in detail [8] and summarized in **Figure 1**.

2.1. Chromium-53 Blood Labeling Procedures

Details of the labelling procedure are in Macallan *et al.* [8]. Briefly, blood 3 - 6 mL was taken from the subject. One mL was stored for analysis of baseline chromium-53 (^{53}Cr) concentration (Pre-labelling blood). The remaining blood was mixed with 0.5 mL solution of ^{53}Cr -labeled hexavalent sodium dichromate. The blood- ^{53}Cr , $\text{Na}_2^{53}\text{Cr}_2\text{O}_7$ mixture was incubated at ambient temperature for 30 min. One mL of this fraction of labeled red cells was retained for analysis (^{53}Cr labeled red cells) and the rest re-injected into the subject. After 30 min, a further 1 mL of sample blood was taken from the subject for the determination of post-labeling ^{53}Cr enrichment (Post-labeling blood) of peripheral blood (**Figure 1**).

An internal standard, a spike of ^{50}Cr -chromium chloride was added to the three sets of samples to quantitatively determine the amounts [8] [14] [15]. Following further processing, chromium-TFA chelate was extracted in hexane, and readied for GC/MS analysis.

2.2. GC/MS Analysis and Mass Isotopomer Calculations

In the original study [8], the analysis of isotope abundance was performed using a GC/MS instrument in electron ionisation (EI) and selective ion monitoring (SIM) mode (*i.e.* GC/MS-EI (SIM)). The dominant ions from the chromium-TFA derivative containing ^{50}Cr , ^{52}Cr and ^{53}Cr isotopes, with the corresponding m/z 356, 358, 359, respectively, were monitored. Relative ion abundances were resolved to yield the relative abundance of natural (most abundant isotope) "N", the "spike" or internal standard chromium, "S" and the "labelled" chromium "L". Samples were run in triplicate.

The original study sort to determine the isotopomer composition of the blood samples, whole unlabeled blood (pre-labeled blood), labeled red blood cells and the sample taken post-injection blood and, blood volume and RBCs lost during the incidence of severe and moderate malaria in children.

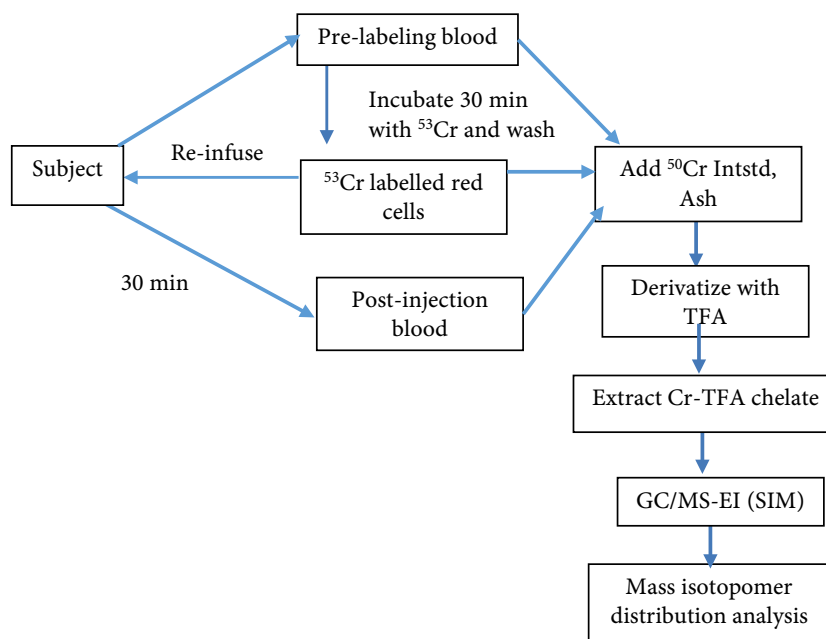


Figure 1. Procedures for labeling RBCs with ^{53}Cr : sampling, labeling, re-infusion, re-sampling, derivatisation, processing and GC/MS (SIM) MIDA analysis.

2.3. Data Handling

The output MS and other data was then deposited into Microsoft Excel and handled in the following steps (see Macallan *et al.* [8], Supplementary Information): The theoretical isotopomer distribution of each mixture (**N**, **S** and **L**) is the sum of two molecular components:

A: The abundance of each chromium isotope is given as (**Table 1(a)**);

a. Natural chromium (%): ^{50}Cr 4.35, ^{52}Cr 83.79, ^{53}Cr 9.5 and ^{54}Cr 2.37 [20].

This is the natural abundance of chromium.

b. The Spike (internal standard, S) chromium (%): ^{50}Cr 96.79, ^{52}Cr 2.98, ^{53}Cr 0.18 and ^{54}Cr 0.05 [14].

c. The label (%): ^{50}Cr 0.0008, ^{52}Cr 1.6, ^{53}Cr 98.23. (^{53}Cr $\text{Na}_2^{53}\text{Cr}_2\text{O}_7$; isotopic purity 98.23%), and

B: The isotopomer distribution of the TFA molecule excludes the chromium ions, assuming the natural isotopic abundances for C, H, O and F (**Table 1(b)**).

The expressions in A (a, b, c) can be written as a system of equations of the form:

$$\text{a. } ax + by + cz$$

$$\text{b. } dx + ey + fz$$

$$\text{c. } gx + hy + iz,$$

where, x , y , z are the measured abundances of the ions corresponding to the isotopes ^{50}Cr , ^{52}Cr and ^{53}Cr , respectively, depending on whether the blood is **N**, **S** or **L**, and a , b , $c \dots i$ are the percent compositions (nine constants). The nine constants together describe the contribution of each mixture to each ion. The composition of ^{54}Cr is included in the data for completeness of the composition data,

but it does not contribute to ions below m/z 360 ($M + 4$). The ^{51}Cr isotope is radioactive and so also makes no contribution. Therefore, a system of equations was derived for A (a, b, c) and then expressed (converting the percent units into decimal units) as a 3×3 square matrix, A (**Table 1(a)**).

$$A = \begin{matrix} & \begin{matrix} 50\text{Cr} & 52\text{Cr} & 53\text{Cr} \end{matrix} \\ \begin{matrix} 50\text{Cr} \\ 52\text{Cr} \\ 53\text{Cr} \end{matrix} & \begin{bmatrix} 0.0435 & 0.8379 & 0.095 \\ 0.9679 & 0.0298 & 0.0018 \\ 0 & 0.016 & 0.9823 \end{bmatrix} \end{matrix} = \begin{matrix} 1.0 \\ 1.0 \\ 1.0 \end{matrix}$$

From **B**, the isotopomer distribution *i.e.* the theoretical proportion of each ion (isotopomers; m/z ^{50}Cr 356, ^{52}Cr 358 and ^{53}Cr 359) of the TFA ($\text{C}_{10}\text{H}_8\text{O}_4\text{F}_6$) molecule but excluding Cr and assuming the natural isotopic abundances for C, H, O and F, was determined from known enrichments (performed separately). This was also expressed as a 3×3 matrix, B (**Table 1(b)**).

Table 1. Calculation of isotope abundances for chromate isotopomers.

(a) Isotopic composition of chromate			
Source of Cr		Proportion	
Isotope	Natural	Spike	Label
50Cr	0.0435	0.9679	0.0008
52Cr	0.8379	0.0298	0.0164
53Cr	0.0950	0.0018	0.9823
54Cr*	0.0237	0.0005	0.0005

(b) Theoretical proportion of each isotopomer generated			
Source of Cr		Proportion	
Isotopomer (m/z)	Natural	Spike	Label
356	0.0385	0.8580	0.0008
358	0.7433	0.0384	0.0145
359	0.1682	0.0055	0.8724

(c) Conversion factors: composition of Cr from ion abundances <i>i.e.</i> inverse matrix of B			
Ion (m/z)	356	358	359
Source of Cr			
Natural	-0.061	1.354	-0.023
Spike	1.168	-0.061	0.000
Label	0.004	-0.261	1.151

Table 1(a) indicates the proportions of Cr isotopes in nature, and the compositions of the spike (internal std) and the label mixtures. The theoretical proportion of each isotopomer generated calculated from known enrichments of natural C/H/O/F in chromate is given in **Table 1(b)**. **Table 1(c)** is the calculated inverse matrix of B (B^{-1}). It gives multiplication factors from which the relative contributions of the three mixtures can be calculated by multiplying with the corresponding raw abundances of the three ions (isotopomers) m/z 356, 358 and 359.

To estimate unknown proportions of chromium isotopic mixtures from the measured abundances of the three ions generated by GC/MS-EI (SIM) from each isotope of chromium when ionised as the TFA-chelate, the inverse matrix of B (B^{-1}) was calculated (**Table 1(c)**). The product of the B^{-1} and the raw MS ion abundances give the true or corrected ion abundances.

Thus, for each subject, the generated MS signal intensities are made up of three treatments: pre-labeling, labeled RBCs and post-label blood, three repetitions each to give nine items in a 3×3 matrix) from mass spectra of the three isotopes, ^{50}Cr , ^{52}Cr and ^{53}Cr -labelled metabolites, and combining them with elements of the 3×3 inverse matrix (B^{-1}), generates the corrected MS data.

The data was handled using *Microsoft Excel*, and the inverse matrix was calculated using the *MINVERSE* function.

2.4. Motivation and Significance

Our motivations for constructing the software package are two-fold; 1) The well established and much used *Microsoft Excel* application has an in-built inverse matrix function, *MINVERSE*, which is suitable for this kind of analysis. However, as a relatively new university (est. 2012) and a new unit, we are determined to have collaborative research within several natural science disciplines: chemical, biological, physics and computational/mathematical sciences. This would enable the harnessing and pooling of scant resources to meet desired needs. Therefore, this short report is on the construction of a database and software tool. The study used chemical data in an earlier study [8] from human sources, application of a matrix and its inverse to answer a physiological question. Therefore, since generating the inverse matrix for the natural abundances of the various elements of TFA is fundamental to the calculation of the corrected ion abundances, we set out to construct an open-source software and data base, *UHAS-MIDA*. The software package would first, handle the 3×3 matrix and generate its inverse. The software package would also receive and manipulate the generated mass isotopomer data, other inputs and, finally, determine the RBC count and fluid volume. 2) To further demonstrate the concept of the matrix as repositories of information and in a chemistry setting. That is, specifically, mass spectrometric data, generated from a biological query can be stored, manipulated mathematically, and lead to providing physiological answers.

We intent to improve on the package to handle a larger set of data in our near-future plans, thus we prepare to conduct a similar study. We envisage that *UHAS-MIDA* would be applicable in labeling metabolic investigations where elements having two or more stable isotopes (e.g. C, O, Ba, Fe, Cr, Cd, Mg, Sr) may be applicable.

In this short report, as a first study, we used the data generated for one subject from Macallan *et al.* [8] (Supplementary material) to construct and illustrate the use of *UHAS-MIDA*. The details of the metadata, software code languages, license, tools, and services used to construct the data base are indicated in **Table 2**.

Table 2. Code metadata and details of the *UHAS-MIDA* software package.

No	Code metadata description	Details
1	Current code version	2022.01
2	Permanent link to code/repository used of this code version	https://github.com/bentil078/Abaye-et-al_UHASmida
3	Code Ocean compute capsule	
4	Legal Code License	MIT license (MIT)
5	Code versioning system used	git
6	Software code languages, tools, and services used	HTML, CSS/Bootstrap, JavaScript and PHP
7	Compilation requirements, operating environments & dependencies	OS: Linux, MacOS or Windows. Dependencies: Apache 2.0+, OpenSSL/1+, MySQL 5.x+ and PHP/5.5+
8	Link to developer documentation & manual	https://github.com/bentil078/Abaye-et-al_UHASmida/tree/main/docs
9	Support email for questions	ebodoom@uhas.edu.gh

3. Results

3.1. Software Architecture and Description

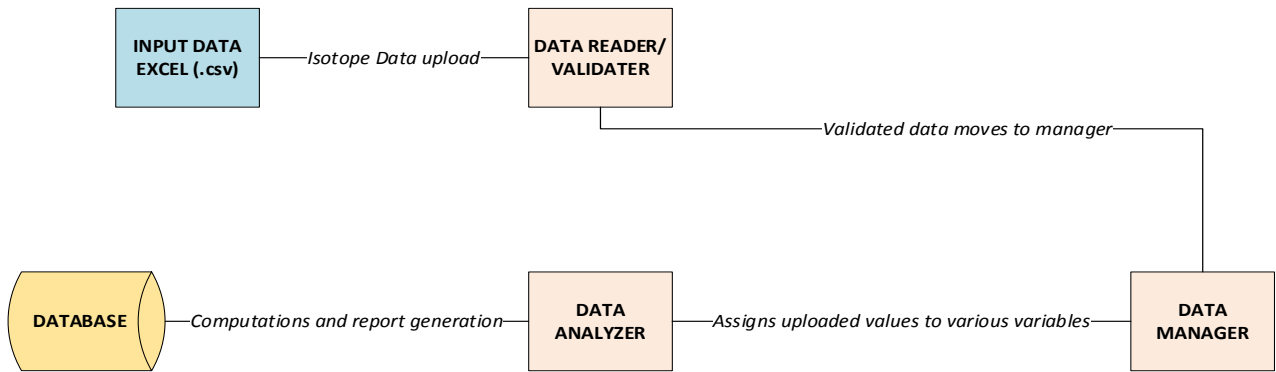
The *UHAS-MIDA* software package was constructed using HTML, CSS/Bootstrap, JavaScript and PHP scripting languages due to the web interface which the application is hosted (**Table 2**). The software includes 1) a database to receive, store and process isotopomer data via a comma-separated values (Excel.csv) file, 2) a parser to upload and translate the data formats, 3) algorithm for handling the matrix (B) and generating its inverse (B^{-1}), 4) an improved application to process and convert the MS signal intensities (**Figure 2(a)**), 5) algorithms to handle the other key inputs; internal standard (^{50}Cr), baseline correction, weight of blood, density of red cells and plasma, in order to calculate 6) the outputs: the volume of blood/kg body wt. and RBC concentration (ng/mL).

As outlined in **Figure 2(b)**, the software package has three main sub-processes *i.e.* 1) Data Input and validation, 2) Data management, and 3) Data analysis. The data input module and validation sub-process allows for either an upload via a .csv file or manual input. The data includes the theoretical isotopomer distribution of each mixture and experimental values (**Table 2(a)**) that are used for the matrix computation and its inverse matrix. The validation module checks for the validity of data being uploaded or entered and returns the user to the start of the application if any error is detected else the process moves to the Data management sub-process. The Data Manager maps the data entered or uploaded to match with their respective placeholders for computation to take place. And, finally, the calculations are executed and the output generated.

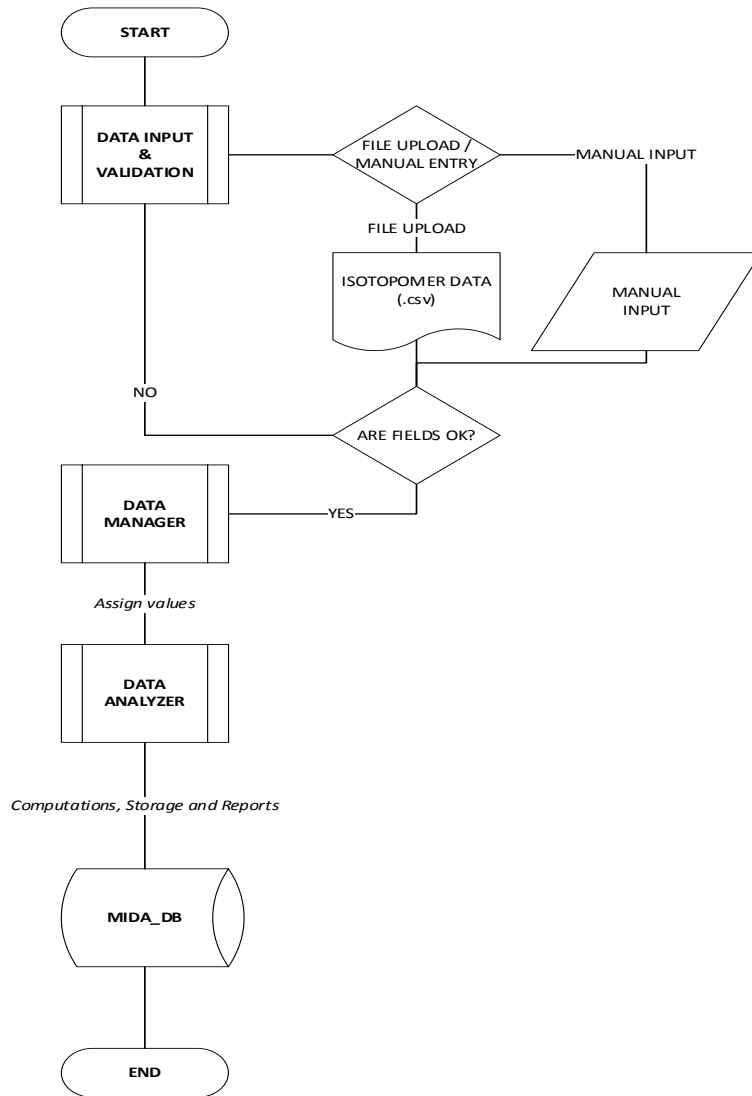
3.2. Software Functionalities

There are a number of previews that are displayed by the software: 1) Baseline Blood Preview, 2) Injected labelled Cells Preview, 3) Post-labelling red blood cells Preview, 4) Blood Volume Preview (Summary Preview). The previews ena-

ble the user to check that the desired steps are well executed before proceeding to the next stage.



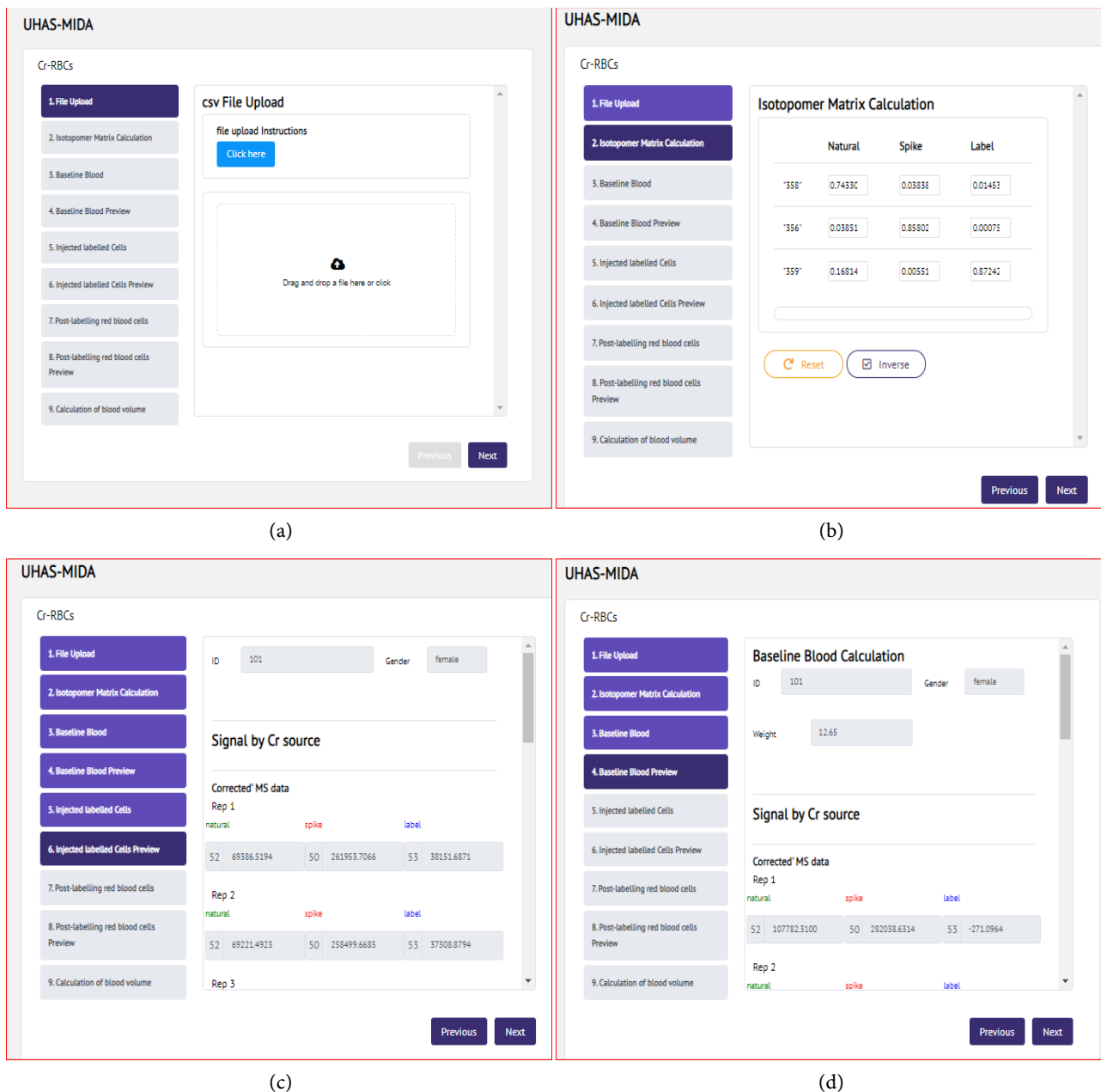
(a)



(b)

Figure 2. (a) The workflow of UHAS-MIDA; (b) Process Flowchart of UHAS-MIDA.

Upon accessing the site, one can download the **.csv file**, and drag and drop, then next click on *Inverse*; this calculates and generates the inverse matrix. And then at “*Raw Mass Data*”, the user is invited to input an ID number for the subject (1, 2, 3....). From the drop down link, the user selects the subject’s gender: male or female. The user then simply follows through to the steps till step 9. These steps are shown in **Figure 3**. In the example given, the subject has the following: ID, 11; gender, female; wt., 12.7 kg; blood haematocrit (Hct) 0.19. The constant attributes for all participants are density of RBCs: 1.095 g/mL; density of plasma: 1.02 g/mL. These are input into the .csv file. The key final outputs are, concentration of RBCs in blood: 4.26 ng/mL; RBC vol: 7.23 mL/kg; blood vol: 45.86 mL/kg (**Figure 3(g)**).



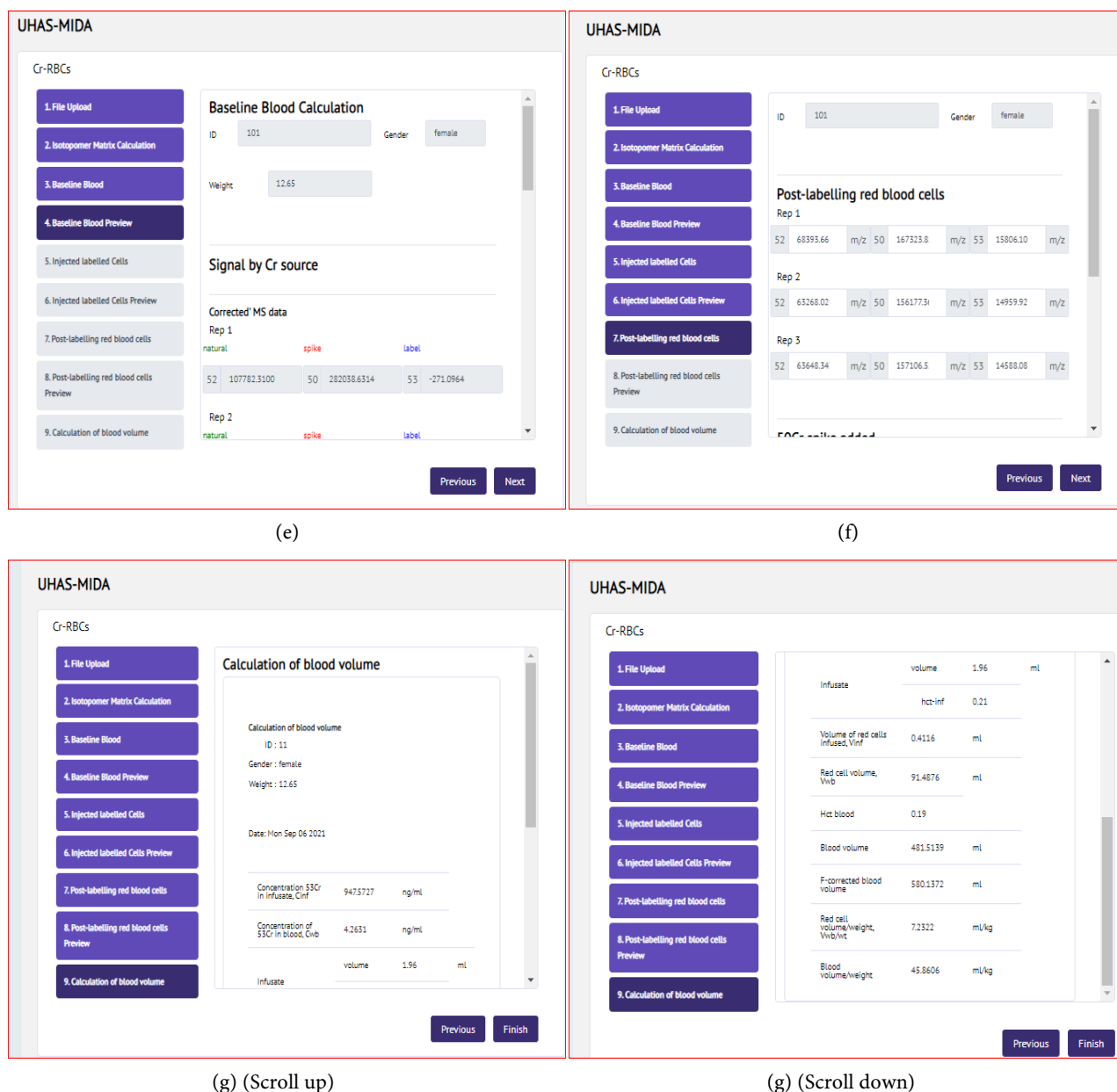


Figure 3. Summary of UHAS-MIDA. Workflow: (a) download and paste .csv file containing the matrix, raw MS ion abundance and other inputs, (b) process the inverse matrix, (c) input the subjects ID number and gender, preview and simply follow on (d), (e), (f). The final output (g) shows the scroll up and scroll down. The key final output (concentration of RBCs in blood: 4.26 ng/mL; RBC vol: 7.23 mL/kg; blood vol: 45.86 mL/kg) is shown in (g).

Thus, *UHAS-MIDA* software package has been designed to be lightweight and this makes it a fast tool. It is well structured and it does not contain website elements such as heavy pictures which would reduce the conversion rate of the software. The package has a simple layout which makes it responsive, usable, and easily accessible.

4. Conclusion

We present *UHAS-MIDA*, a HTML, CSS/Bootstrap, JavaScript, and PHP-based

utility handling stable isotope MS ion abundance data, which calculates the rate of red blood cell and fluid volume loss during malaria in children. The software package generates a 3×3 matrix and its inverse as part of the key steps in the process. The 3×3 matrix is as wieldy as the *Microsoft* “*MINVERSE*” functions, displaying all the elements in the inverse matrix. The study demonstrates an application of a matrix and its inverse in a biological setting in answering a chemical query. We discuss the potential of *UHAS-MIDA* to be useful and applicable in labeling investigations where elements having two or more stable isotopes are involved in the metabolic process and present an illustrative example. Here, we provide this software tool as a first version and intend to build upon it with more MS data. It is also available to the wider metabolic, mass spectrometry, and bioinformatics community.

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Authors' Contributions

The concept was envisioned by Daniel A. Abaye and Irene A. Agbo. The software code was implemented by Emmanuel B. Odoom and Samuel Agana. The database construction, data curation and formal analysis were done by Emmanuel B. Odoom, John-Bosco Diekuu, Ernest Y. Boateng and Samuel Agana. The writing of the manuscript was led by Daniel A. Abaye. All authors contributed to the writing of the original manuscript. All authors contributed and approved the final manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

- [1] Clapperton, A.T., Coward, W.A. and Bluck, L.J.C. (2002) Measurement of Insulin Sensitivity Indices Using ^{13}C -Glucose and Gas Chromatography/Combustion/Isotope Ration Mass Spectrometry. *Rapid Communications in Mass Spectrometry*, **16**, 2009-2014. <https://doi.org/10.1002/rcm.815>
- [2] Bluck, L.J., Izzard, A.P. and Bates, C.J. (1996) Measurement of Ascorbic Acid Kinetics in Man Using Stable Isotopes and Gas Chromatography/Mass Spectrometry. *Journal of Mass Spectrometry*, **31**, 741-748. [https://doi.org/10.1002/\(SICI\)1096-9888\(199607\)31:7%3C741::AID-JMS352%3E3.0.CO;2-H](https://doi.org/10.1002/(SICI)1096-9888(199607)31:7%3C741::AID-JMS352%3E3.0.CO;2-H)
- [3] Bluck, L.J., Jones, K.S. and Bates, C.J. (2002) ^2H - and ^{13}C -Labelled Tracers Compared for Kinetic Studies of Ascorbic Acid Metabolism in Man: A Factor Analytical Approach. *Rapid Communications in Mass Spectrometry*, **16**, 879-883. <https://doi.org/10.1002/rcm.653>

- [4] Ong, S.E., Blagoev, B., Kratchmarova, I., Kristensen, D.B., Steen, H., Pandey, A. and Mann, M. (2002) Stable Isotope Labeling by Amino Acids in Cell Culture, SILAC, as a Simple and Accurate Approach to Expression Proteomics. *Molecular and Cell Proteomics*, **1**, 376-386. <https://doi.org/10.1074/mcp.m200025-mcp200>
- [5] Abaye, D.A., Morrison, D.J. and Preston, T. (2011) Strong Anion Exchange Liquid Chromatographic Separation of Protein Amino Acids for Natural ^{13}C -Abundance Determination by Isotope Ratio Mass Spectrometry. *Rapid Communications in Mass Spectrometry*, **25**, 429-435. <https://doi.org/10.1002/rcm.4844>
- [6] Godin, J.P., Mermoud, A.F., Rémond, D., Faure, M., Breuille, D., Williamson, G., Peré-Trepat, E., Ramadan, Z., Fay, L.B. and Kochhar, S. (2009) Simultaneous Measurement of ^{13}C - and ^{15}N -Isotopic Enrichments of Threonine by Mass Spectrometry. *Rapid Communications in Mass Spectrometry*, **23**, 1109-1115. <https://doi.org/10.1002/rcm.3976>
- [7] Planche, T., Onanga, M., Schwenk, A., Dzeing, A., Borrmann, S., Faucher, J.F., Wright, A., Bluck, L., Ward, L., Kombila, M., Kremsner, P.G. and Krishna, S. (2004) Assessment of Volume Depletion in Children with Malaria. *PLoS Medicine*, **18**, e18. <https://doi.org/10.1371/journal.pmed.0010018>
- [8] Macallan, D.C., Abaye, D.A., Dottin, S., Onanga, M., Kombila, M., Dzeing-Ella, A., Kremsner, P.G., Krishna, S. and Planche, T. (2009) Blood Volume and Red Cell Mass in Children with Moderate and Sever Malaria Measured by Chromium-53 Dilution and Gas Chromatography/Mass Spectrometric Analysis. *Rapid Communications in Mass Spectrometry*, **23**, 2467-2475. <https://doi.org/10.1002/rcm.4058>
- [9] Dauner, M., Bailey, J. and Sauer, U. (2001) Metabolic Flux Analysis with Comprehensive Isotopomer Model in *Bacillus subtilis*. *Biotechnology and Bioengineering*, **76**, 144-156. <https://doi.org/10.1002/bit.1154>
- [10] Wiechert, W. (2001) ^{13}C Metabolic Flux Analysis. *Metabolic Engineering*, **3**, 195-206. <https://doi.org/10.1006/mben.2001.0187>
- [11] Zamboni, N., Fischer, E. and Sauer, U. (2005) FiatFlux—A Software for Metabolic Flux Analysis from ^{13}C -Glucose Experiments. *BMC Bioinformatics*, **6**, Article No. 209. <https://doi.org/10.1186/1471-2105-6-209>
- [12] Hellerstein, M.K. and Neese, R.A. (1992) Mass Isotopomer Distribution Analysis: A Technique for Measuring Biosynthesis and Turnover of Polymers. *American Journal of Physiology*, **263**, 988-1001. <https://doi.org/10.1152/ajpendo.1992.263.5.e988>
- [13] Rousu, J., Rantanen, A., Ketola, R.A. and Kokkonen, J.T. (2005) Isotopomer Distribution Computation from Tandem Mass Spectrometric Data with Overlapping Fragment Spectra. *Spectroscopy*, **19**, 53-67. <https://researchportal.helsinki.fi/en/publications/isotopomer-distribution-computation-from-tandem-mass-spectrometry>
- [14] Veillon, C., Patterson, K.Y., Nagey, D.A. and Tehan, A.M. (1994) Measurement of Blood Volume with an Enriched Stable Isotope of Chromium (^{53}Cr) and Isotope Dilution by Combined Gas Chromatography-Mass Spectrometry. *Clinical Chemistry*, **40**, 71-73. <https://doi.org/10.1093/clinchem/40.1.71>
- [15] Silver, H.M., Seebeck, M.A. and Carlson, R. (1998) Comparison of Total Blood Volume in Normal, Preeclamptic, and Nonproteinuric Gestational Hypertensive Pregnancy by Simultaneous Measurement of Red Blood Cell and Plasma Volumes. *American Journal of Obstetrics and Gynecology*, **179**, 87-96. [https://doi.org/10.1016/S0002-9378\(98\)70255-8](https://doi.org/10.1016/S0002-9378(98)70255-8)
- [16] Karlstaedt, A. (2021) Stable Isotopes for Tracing Cardiac Metabolism in Diseases. *Frontiers in Cardiovascular Medicine*, **8**, Article ID: 734364.

- <https://doi.org/10.3389/fcvm.2021.734364>
- [17] Ahmed, Z., Zeeshan, S., Huber, C., Hensel, M., Schomburg, D., Münch, R., Eisenreich, W. and Dandekar, T. (2013) Software LC-MIDA for Efficient Mass Isotopomer Distribution Analysis in Metabolic Profiling. *BMC Bioinformatics*, **14**, Article No. 218. <https://doi.org/10.1186/1471-2105-14-218>
- [18] Ahmed, Z., Zeeshan, S., Huber, C., Hensel, M., Schomburg, D., Münch, R., Eylert, E., Eisenreich, W. and Dandekar, T. (2014) 'Isotopo' a Database Application for Facile Analysis and Management of Mass Isotopomer Data. *Database*, **2014**, bau077. <https://doi.org/10.1093/database/bau077>
- [19] Brauman, J.I. (1966) Least Squares Analysis and Simplification of Multi-Isotope Mass Spectra. *Analytical Chemistry*, **38**, 607-610. <https://pubs.acs.org/doi/abs/10.1021/ac60236a020>
<https://doi.org/10.1021/ac60236a020>
- [20] Rosman, K.J.R. and Taylor, P.D.P. (1998) Isotopic Composition of the Elements 1997 (Technical Report). *Pure and Applied Chemistry*, **70**, 217-235. <http://publications.iupac.org/pac/70/1/0217/index.html>
<https://doi.org/10.1351/pac199870010217>