



HAL
open science

Measurement of S100B protein: evaluation of a new prototype on a bioMérieux Vidas® 3 analyzer.

Charlotte Oris, Russel Chabanne, Julie Durif, Samy Kahouadji, Marina Brailova, Vincent Sapin, Damien Bouvier

► To cite this version:

Charlotte Oris, Russel Chabanne, Julie Durif, Samy Kahouadji, Marina Brailova, et al.. Measurement of S100B protein: evaluation of a new prototype on a bioMérieux Vidas® 3 analyzer.. *Clinical Chemistry and Laboratory Medicine*, 2019, 57 (8), pp.1177-1184. 10.1515/cclm-2018-1217 . hal-03023298

HAL Id: hal-03023298

<https://uca.hal.science/hal-03023298>

Submitted on 3 Jun 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

1 **Measurement of S100B protein:**

2 **evaluation of a new prototype on a bioMérieux Vidas® 3 analyzer**

3 Charlotte Oris¹, Russel Chabanne², Julie Durif¹, Samy Kahouadji¹, Marina Brailova¹,

4 Vincent Sapin^{1,3}, Damien Bouvier^{1,3}

5
6 **Affiliations:**

7 ¹Biochemistry and Molecular Biology Department, CHU Clermont-Ferrand, Clermont-
8 Ferrand, France; ²Department of Perioperative Medicine, CHU Clermont-Ferrand, Clermont-
9 Ferrand, France; ³Clermont Auvergne University, CNRS, INSERM, GReD, Clermont-
10 Ferrand, France.

11
12 **Short title:** Evaluation of a new S100B assay.

13
14 **Corresponding author:** Damien Bouvier (MD-PhD), Service de Biochimie Médicale, Centre
15 de Biologie, CHU Gabriel Montpied, 58 Rue Montalembert, 63000 Clermont-Ferrand, France
16 Tel.: + 33 4 73 75 48 82 / Fax: + 33 4 73 75 18 55 / Email: dbouvier@chu-clermontferrand.fr.

17
18 **Word count:** 2933.

19
20 **Number of tables and figures:** 9.

1 **Abstract**

2 **Background:** The addition of S100B protein to guidelines for the management of mild
3 traumatic brain injury (mTBI) decreases the amount of unnecessary computed tomography
4 scans with a significant decrease in radiation exposure and an increase in cost savings. Both
5 DiaSorin and Roche Diagnostics have developed automated assays for S100B determination.
6 Recently, bioMérieux developed a prototype immunoassay for serum S100B determination.
7 For the first time, we present the evaluation of the S100B measurement using a bioMérieux
8 Vidas[®] 3 analyzer.

9 **Methods:** We evaluated the matrix effects of serum and plasma, and their stability after
10 storage at 2-8°C, -20°C, and -80°C. The new measurement prototype (bioMérieux) was
11 compared with an established one (Roche Diagnostics), and a precision study was also
12 conducted. Lastly, clinical diagnostics performance of the bioMérieux and Roche Diagnostics
13 methods were compared for 80 patients referred to the Emergency Department for mTBI.

14 **Results:** Stability after storage at 2-8°C, -20°C, and -80°C and validation of the serum matrix
15 were demonstrated. The bioMérieux analyzer was compared to the Roche Diagnostics system,
16 and the analytical precision was found to be efficient. Clinical diagnosis performance
17 evaluation confirmed the predictive negative value of S100B in the management of mTBI.

18 **Conclusions:** The study's data are useful for interpreting serum S100B results on a
19 bioMérieux Vidas[®] 3 analyzer.

20 **Keywords:** S100B, Cobas, Vidas, sample stability, assay comparison

21

1 **List of abbreviations:**

2 CT: Computed Tomography

3 CV: Coefficient of Variation

4 ED: Emergency Department

5 GCS: Glasgow Coma Scale

6 IQR: InterQuartile Range

7 Max: maximum

8 Min: minimum

9 mTBI: mild Traumatic Brain Injury

10 NCCU: Neuro-Critical Care Unit

11 SD: Standard Deviation

12 SFMU: French Society of Emergency Medicine

13

14

15

1 **1. Introduction**

2 Computed tomography (CT) is the gold standard diagnostic tool for diagnosing mild
3 traumatic brain injury (mTBI). However, the use of CT scans is limited due to radiations risks
4 (1–3) and economic implications (4). Moreover, most CT scans could be avoided because
5 only a small number of mTBI patients have intracerebral lesions (5). An alternative strategy to
6 using CT scan has been developed based on blood protein biomarkers of brain damage, and
7 S100B protein has emerged as a candidate biomarker in children and adults (6,7). The
8 addition of S100B detection to the Scandinavian guidelines for the management of mTBI
9 decreased the need for CT scans among adults by one-third, using a cut-off of 0.10 µg/L (8).
10 S100B protein is one of the calcium binding proteins found in glial cells. It is a small dimeric
11 cytosolic protein (21 kDa) consisting of ββ or αβ chains. It is involved in a variety of
12 intracellular and extracellular regulatory activities (9,10). Because S100B has a short half-life,
13 a maximal interval of 3 hours is recommended between trauma and blood sampling (11).

14 Both DiaSorin and Roche Diagnostics have developed automated assays for serum
15 S100B protein determination. In comparison to manual assays, automated assays provide
16 better analytical results with regard to precision, linearity, and accuracy, and they seem to be a
17 preferable option for S100B measurement (12,13). On a pre-analytical level, only one study
18 has evaluated and compared the matrix effect between plasma and serum (14). A few studies
19 have evaluated the stability of S100B after storage at room temperature or at 4°C (15,16).
20 However, the stability in frozen samples has never been clearly identified in the literature. On
21 an analytical level, the precision (12,13) of DiaSorin and Roche Diagnostics S100B assays as
22 well as a comparison (11–13,17,18) of both methods have been reported. With regard to
23 diagnostics clinical performance, it is essential that the performance of S100B assays are
24 comparable in order to reduce the use of CT scans after mTBI.

1 The clinical utility of S100B protein has attracted new companies that specialize in *in*
2 *vitro* diagnosis. One company, bioMérieux, has developed an automated prototype for S100B
3 protein assay on a Vidas[®] 3 analyzer. To date, no study has evaluated this new technique. For
4 the first time, we set out to evaluate the pre-analytical (matrix effect of serum and plasma, and
5 their stability at 2-8°C, -20°C, and -80°C), analytical (imprecision and comparison), and
6 diagnostics clinical performance in context of mTBI (CT scans reduction in adults with
7 mTBI) of the bioMérieux S100B prototype assay.

8 **2. Materials and methods**

9 **2.1 Study design and patients**

10 This study was conducted at the Clermont-Ferrand teaching hospital and in accordance
11 with the Declaration of Helsinki principles for ethical medical research involving human
12 subjects. The project was also approved by the institutional ethic review board of the hospital.
13 Patients and their families were informed of their right to express their disagreement
14 regarding the use of their biological samples. Venous blood samples were taken from 71
15 patients from the Neuro-Critical Care Unit (NCCU) and 80 patients from the Emergency
16 Department (ED). All study subjects were ≥ 18 years old. All patients in NCCU (referred in
17 different contexts as subarachnoid hemorrhage, neurosurgical postoperative ...) benefit from
18 an S100B assay in their first biological assessment and were included only to evaluate the
19 matrix effect of serum and plasma, the stability after storage at 2-8°C, -20°C, -80°C and the
20 comparison between bioMérieux and Roche Diagnostics assays (Table 1). Three groups of
21 S100B ranges of equal sizes were formed: ≤ 0.10 $\mu\text{g/L}$; 0.11 to 0.50 $\mu\text{g/L}$; > 0.50 $\mu\text{g/L}$ for
22 their clinical interest. The concentration of 0.10 $\mu\text{g/L}$ corresponds to the worldly used
23 decision threshold of patients with mTBI (19) whereas concentrations greater than of 0.50
24 $\mu\text{g/L}$ is relevant for moderate to severe injury (20). Patients from ED were included for the
25 comparison between bioMérieux and Roche assays and the study of clinical diagnostics

1 performance in context of mTBI (Table 1). Patient samples from the ED were drawn into
2 serum gel separator tubes (tube a). Samples from patients in the NCCU (n = 44) were drawn
3 into tube a, serum separator tubes without gel (tube b), and lithium heparinate gel separator
4 tubes (tube c). For the other 27 patients from the NCCU, the samples were drawn into tube a
5 and tube c.

6 **2.2 S100B assay**

7 Samples were sent to the laboratory for processing within one hour, then they were
8 centrifuged (2200 g, 15 min). S100B concentrations were determined using an
9 electrochemiluminescence immunoassay on a Cobas e411[®] analyzer (Roche Diagnostics,
10 Meylan, France) and an automated enzyme-linked fluorescence assay on a Vidas[®] 3 analyzer
11 (bioMérieux, Marcy l'Etoile, France). The lower limit of detection is 0.005 µg/L for the Roche
12 Diagnostics assay and 0.012 µg/L for the bioMérieux assay. The test result is available in 18
13 minutes for the Roche Diagnostics assay and 20 minutes for the bioMérieux assay. Both
14 assays detect S100 dimers that contain S100B (S100BB and S100A1B). For bioMérieux
15 assay, one calibrator was used for every 28-days recalibration. For Roche Diagnostics assay, 2
16 calibrators were used for every 3-months recalibration. For both assays, two control levels
17 were used.

18 **2.3 Matrix effect and storage stability**

19 Blood samples from 44 were collected using three different tubes: tube a, tube b, and
20 tube c. The samples were analyzed immediately after centrifugation (T0). Then, the samples
21 were stored 24 h (8 h at room temperature and 16 h at 2-8°C) and analyzed (T24). The
22 samples were again stored at 2-8°C for 24 h and reanalyzed (T48) (Figure 1A).

23 Blood samples from 71 subjects were collected using two different tubes: tube a and
24 tube c. The samples were analyzed immediately after centrifugation, and they were then

1 divided into 5 aliquots to measure the S100B concentration after storage at -20°C (for 1, 3,
2 and 6 months) and -80°C (for 3 and 9 months) (Figure 1B).

3 **2.4 Precision and comparison of the methods**

4 The repeatability (n = 30 in 1 day) and reproducibility (n = 30 over 15 days) were
5 determined for the Vidas® 3 analyzer S100B assay using two quality controls (ref #415739)
6 and two laboratory-made serum pools at clinically-relevant decision levels, including pools
7 for the median and highly pathological range within the reference range. For each pool, 30
8 aliquots were prepared and frozen at -80°C. For the measurements (made twice daily over 15
9 days), the aliquots were freshly defrosted, centrifuged, and measured.

10 For comparison, parallel measurements were performed in the 151 serum samples
11 obtained from the patients (80 from the ED and 71 from the NCCU) using both Roche
12 Diagnostics Cobas e411® and bioMérieux Vidas® 3 analyzers.

13 **2.5 Clinical diagnostics performance in context of mTBI**

14 Eighty patients admitted to the ED with mTBI were enrolled in this study. Inclusion
15 criteria for the use of S100B determination in mTBI management were: ≥ 18 years of age,
16 blood sample draw performed within 3 h after trauma, an mTBI with a medium risk of
17 complications (i.e., with a Glasgow Coma Scale [GCS] equal to 15 and antiplatelet treatment
18 or loss of consciousness or retrograde amnesia 30 min after injury) (21). Exclusion criteria
19 were: an mTBI with a low risk of complications (i.e., an asymptomatic patient without
20 medium- or high-risk criteria and a GCS of 15), an mTBI with a high risk of complications
21 (i.e., a GCS < 15 within 2 h post-injury, or focal neurology, post-traumatic convulsion, open
22 skull fracture, embarrassment, signs of skull base fracture, anticoagulant intake, or repeated
23 vomiting) (21).

24 At the Clermont-Ferrand ED, S100B serum is measured on a Roche Diagnostics
25 Cobas e411® assay and integrated into a decision algorithm from the French Society of

1 Emergency Medicine (SFMU) to manage patients with mTBI (21). If the S100B value is
2 $<0.10 \mu\text{g/L}$, a CT scan is not prescribed; however, a CT scan is recommended if the S100B
3 value is $\geq 0.10 \mu\text{g/L}$ (21). In our study, serum S100B concentrations were determined using a
4 Roche Diagnostics Cobas e411[®] assay (value communicated to the physician) and
5 immediately measured on a Vidas[®] 3 bioMérieux assay (value not communicated to the
6 physician). The same cut-off ($0.10 \mu\text{g/L}$) was used for both assays. Clinical data were
7 collected for each patient (CT scan prescription or no CT scan prescription, normal CT scan
8 or CT scan with intracerebral lesion).

9 **2.6 Statistics**

10 Statistical analysis was performed using SAS 9.1.3 software (SAS Institute, Cary, NC,
11 USA). For quantitative data, normality was verified using the Shapiro–Wilk test. Data were
12 presented as median (minimum [min] and maximum [max], interquartile range [IQR]), as it
13 was not normally distributed. Comparisons of the S100B median concentrations (for the
14 matrix effect and stability study) were interpreted using the Kruskal-Wallis test followed by
15 the Dunn post-test. A *P* value <0.05 was considered to be statistically significant. To study
16 the clinical performance for patients admitted to the ED, differences in the positive and
17 negative S100B values between the Roche Diagnostics Cobas e411[®] and the bioMérieux
18 Vidas[®] 3 assays were compared using McNemar’s test. Then, a Fisher’s test was used to
19 compare the sub-categories (CT scan prescription, no CT scan prescription). A comparison of
20 the Roche Diagnostics and bioMérieux methods was performed using linear regression and a
21 Bland-Altman plot. The Bland-Altman plot was also used to compare the serum S100B levels
22 (tube a) and plasma S100B concentrations (tube c).

23 **3. Results**

24 Patient demographics were summarized in Table 1.

3.1 Matrix effect and storage stability

At T0, the S100B median concentrations determined in the tube a samples, the tube b samples, and the tube c samples (plasma) were 0.20 µg/L (min: 0.03; max: 4.37; EI: 0.08–0.56), 0.20 µg/L (min: 0.03; max: 4.59; EI: 0.08–0.54), and 0.23 µg/L (min: 0.05; max: 4.77; EI: 0.10–0.61), respectively. The difference between these three medians was not statistically significant ($p = 0.49$). However, the Bland-Altman plot showed higher values for the plasma concentrations. The mean of the differences between the tube c samples and the tube a samples was 0.07 µg/L (Figure 2). Global overestimation of the plasma concentrations was approximately 20%.

For samples of tubes a, b, and c, the median concentrations determined at T0, T24, and T48 are summarized in Table 2. No significant difference was identified, between the three times, for tube a samples ($p = 0.83$), tube b samples ($p = 0.95$), and tube c samples ($p = 0.56$) (Table 2).

No statistically significant difference was found between the median concentrations of S100B after storage at -20°C (Table 3) or at -80°C (Table 4).

3.2 Precision and comparison of the methods

The coefficients of variation of repeatability and reproducibility were summarized in Table 5 for quality controls and laboratory-made serum pools. Repeatability was evaluated by 30 repeated measurements of the same sample whereas reproducibility was evaluated by 30 measurements taken over 15 days.

The correlation between the bioMérieux Vidas[®] 3 and Roche Diagnostics Cobas e411[®] methods for the S100B values ($n=151$) was: $r=0.97$, slope 1.13, intercept 0.002 (Figure 3A). The Bland-Altman plot showed a mean difference between the two methods of -0.06 µg/L (Figure 3B).

3.3 Clinical diagnostics performance in context of mTBI

S100B measurement was indicated for the management of each of the 80 patients with mTBI admitted to the ED. The median age was 42 years (min: 18; max: 91; EI: 21 – 65) and the sex ratio male/female was 1.58 (Table 1). S100B concentrations were measured using both the Roche Diagnostics and bioMérieux methods. The percentages of negative S100B (<0.10 µg/L) were 40% and 37.5%, for the Roche Diagnostics and bioMérieux methods, respectively. No statistically significant difference was found between these two techniques ($p = 0.41$) (Table 6). For the three patients with intracerebral lesions on CT scan, the S100B concentrations were positive (≥ 0.10 µg/L) with the Roche Diagnostics and bioMérieux methods. The ability of S100B to identify intracerebral lesions was not significantly different between the two analyzers ($p = 0.53$) (Table 6). The physicians did not use the decision algorithm for 10% of the patients. For 5% of the patients, a CT scan was prescribed despite a negative S100B. For an additional 5% of the patients, a CT scan was not prescribed, even though the S100B values were positive (Table 6).

4. Discussion

Currently, two companies specialize in *in vitro* diagnosis: Roche Diagnostics and DiaSorin. Both companies offer automated analyzers that can determine the S100B protein concentration in serum. For the first time, the present study evaluated the analytical performances of an automated prototype for an S100B protein assay: the bioMérieux Vidas[®] 3. It should be noted that the Vidas[®] analyzer is the most widely used immunoassay system in clinical laboratories, worldwide. Compared with the Cobas[®] analyzer, the Vidas[®] offers several advantages such as the fast adoption (easier-to-use assay), the cost avoidance in low throughput samples (1 test for 1 patient), the random access with up to 4 independent sections and no daily maintenance.

1 It is essential to obtain data on the influence of the matrix effect and storage in order to
2 avoid pre-analytical errors that may affect the interpretation of the test result. Evaluation of
3 the matrix effect showed an overestimation of $\approx 20\%$ for the plasma values in comparison to
4 the serum values. This difference was also reported by Tort *et al.* (14). Two other publications
5 evaluated the impact of heparin treatment on S100B measurement (22,23). Wang *et al.*
6 showed a rapid increase in serum S100B (1.74 fold) within 15 min of unfractionated heparin
7 administration (1.74 fold) and 3 h after low molecular weight heparin injection (1.44 fold)
8 (23). While heparin constantly appears to increase the immuno-reactivity for S100B, the
9 presence of the other anticoagulants in blood samples interferes chaotically with the S100B
10 measurement. Indeed, ethylenediaminetetraacetic acid (EDTA) and citrate are calcium
11 chelators, whereas heparin action is independent of calcium (14). Our stability study showed
12 no difference between the results of the S100B measurements after storing the samples at
13 room temperature for 8 h and at 2–8°C for 48 h. Our findings are in agreement with Raabe *et*
14 *al.* who demonstrated that storage at 4°C for 48 h did not affect the measurement result (15).
15 In contrast, Djukanovic *et al.* showed that storing samples at room temperature after 3 h was
16 associated with an increase in the S100B values (16). In terms of S100B measurement after
17 freezing, we demonstrated, for the first time, stability after storing the samples at -20°C for 6
18 months and at -80°C for 9 months. However, three plasma samples showed unsatisfactory
19 storage with post-freeze concentrations 7- to 10-times higher than initial concentrations.
20 Knowing that S100B is expressed in lymphocyte cells (13,24), we hypothesized that a release
21 of protein after lysis was caused by freezing of residual lymphocytes. Indeed, despite using a
22 separator gel, a significant proportion of the cells (leukocytes, red blood cells, and platelets)
23 were found in the plasma (25).

24 The precision study (repeatability and reproducibility) results demonstrated that the
25 bioMérieux method was very satisfactory, which is consistent with the data reported by the

1 manufacturer. The within run CVs (1.7–2.2%) and between run CVs (2.8–3.9%) of the
2 bioMérieux method were less than the CVs of the DiaSorin method (within run CV: 4.0–
3 5.6%; between run CVs: 3.7–6.1%), and they were very similar to those of the Roche
4 Diagnostics method (within run CV: 1.1–2.0%; between run CVs: 1.8–2.1%) (13). Therefore,
5 the precision of the Vidas[®] 3 S100B prototype is comparable to the two analyzers that are
6 routinely used in clinical settings.

7 Moreover, the bioMérieux and Roche Diagnostics methods showed good correlation
8 ($r=0.97$) for S100B protein determination. However, the two methods were not
9 interchangeable; the mean values were 13% higher with the bioMérieux assay. Moreover, the
10 gap between these two techniques widened as the S100B concentrations increased. Overall,
11 the results of this study are in line with the findings reported in the literature; that is, the
12 results of these two immunoassays are correlated, but not interchangeable. In the literature,
13 comparisons of the Roche Diagnostics and DiaSorin methods showed an overestimation close
14 to 30% for the DiaSorin assay (11–13,17,18). However, the three methods detect S100 dimers
15 that contain S100B (S100BB and S100 A1B). The use of a single S100B protein assay
16 technique is essential for patient management and follow-up. However, currently, no
17 diagnostic test is registered for patient follow-up. The absence of a standardized S100
18 immunoassay could clearly explain the observed difference reported in the literature.

19 In our study, the addition of S100B to the SFMU's algorithm could reduce the use of
20 CT scans by 40% and 37.5% for the Roche Diagnostics and bioMérieux assays, respectively.
21 This better specificity than that usually described (33%) is probably due to an age effect.
22 Indeed, in our study, the proportion of people over 65 years is lower than in a previous study
23 showing that the specificity is lower on this age group (21).

24 There are some limitations to our study. The cutoff of 0.10 $\mu\text{g/L}$ was evaluated for the
25 management of adults with mTBI ($n = 80$). However, interventional studies on larger cohorts

1 are required. Moreover, it would be interesting to study S100B in other indications than mTBI
2 like subarachnoid hemorrhage and severe trauma. Reference ranges of serum S100B
3 concentration should also be made for children as for Roche Diagnostics (26) and DiaSorin
4 (13) technology.

5 In conclusion, this study provided valuable new data for the concerted interpretation of
6 S100B assay results by biologists and clinicians. Pre-analytical stability and validation of
7 serum matrix were demonstrated. The bioMérieux analyzer was compared to the Roche
8 Diagnostics system. The precision study results for the bioMérieux Vidas[®] 3 analyzer showed
9 that this method had very satisfactory results. Clinical diagnostics performance confirmed the
10 predictive negative value of the S100B biomarker in the management of mTBI in adults.

11

12

13

14

15

1 **Acknowledgements**

2 We thank the Emergency Department and the Neurological Intensive Care Unit at Clermont-
3 Ferrand Hospital for their help in specimen collection. We wish to acknowledge the excellent
4 technical assistance of Laure Allard, Frédérique Raymond and Corinne Perret.

5 **Author contributions**

6 All the authors have accepted responsibility for the entire content of this submitted manuscript
7 and approved submission.

8 **Research funding**

9 This work was supported by the bioMérieux company.

10 **Employment or leadership**

11 None declared.

12 **Honorarium**

13 None declared.

14 **Competing interests**

15 None declared.

16

17

18

1 **References**

- 2 1. Brenner DJ. Computed Tomography An Increasing Source of Radiation Exposure. *N Engl*
3 *J Med* 2007;357:2277–84.
4
- 5 2. Mathews JD, Forsythe AV, Brady Z, Butler MW, Goergen SK, Byrnes GB, et al. Cancer
6 risk in 680 000 people exposed to computed tomography scans in childhood or adolescence:
7 data linkage study of 11 million Australians. *BMJ* 2013;346:f2360.
8
- 9 3. Pearce MS, Salotti JA, Little MP, McHugh K, Lee C, Kim KP, et al. Radiation exposure
10 from CT scans in childhood and subsequent risk of leukaemia and brain tumours: a
11 retrospective cohort study. *Lancet* 2012;380:499–505.
12
- 13 4. Calcagnile O, Anell A, Undén J. The addition of S100B to guidelines for management of
14 mild head injury is potentially cost saving. *BMC Neurol* 2016;16:200.
15
- 16 5. Stiell IG, Wells GA, Vandemheen K, Clement C, Lesiuk H, Laupacis A, et al. The Canadian
17 CT Head Rule for patients with minor head injury. *Lancet* 2001;357:1391–6.
18
- 19 6. Undén J, Romner B. Can low serum levels of S100B predict normal CT findings after
20 minor head injury in adults?: an evidence-based review and meta-analysis. *J Head Trauma*
21 *Rehabil* 2010;25:228–40.
22
- 23 7. Oris C, Pereira B, Durif J, Simon-Pimmel J, Castellani C, Manzano S, et al. The Biomarker
24 S100B and Mild Traumatic Brain Injury: A Meta-analysis. *Pediatrics* 2018;141:e20180037.
25
- 26 8. Undén L, Calcagnile O, Undén J, Reinstrup P, Bazarian J. Validation of the Scandinavian
27 guidelines for initial management of minimal, mild and moderate traumatic brain injury in
28 adults. *BMC Med* 2015;13:292.
- 29 9. Zimmer DB, Cornwall EH, Landar A, Song W. The S100 protein family: history, function,
30 and expression. *Brain Res Bull* 1995;37:417–29.
31
- 32 10. Donato R. S100: a multigenic family of calcium-modulated proteins of the EF-hand type
33 with intracellular and extracellular functional roles. *Int J Biochem Cell Biol* 2001;33:637–68.
- 34 11. Laribi S, Kansao J, Borderie D, Collet C, Deschamps P, Ababsa R, et al. S100B blood
35 level measurement to exclude cerebral lesions after minor head injury: the multicenter STIC-
36 S100 French study. *Clin Chem Lab Med* 2014;52:527–36.
- 37 12. Smit LHM, Korse CM, Bonfrer JMG. Comparison of four different assays for
38 determination of serum S-100B. *Int J Biol Markers* 2005;20:34–42.
- 39 13. Bouvier D, Duret T, Rouzair P, Jabaudon M, Rouzair M, Nourrisson C, et al.
40 Preanalytical, analytical, gestational and pediatric aspects of the S100B immuno-assays. *Clin*
41 *Chem Lab Med* 2016;54:833–42.
- 42 14. Tort ABL, Dietrich MO, Gonçalves CA, Souza DO, Portela LVC. Influence of
43 anticoagulants on the measurement of S100B protein in blood. *Clin Biochem* 2003;36:629–
44 32.

- 1 15. Raabe A, Kopetsch O, Groß U, Zimmermann M, Gebhart P. Measurements of Serum S-
2 100B Protein: Effects of Storage Time and Temperature on Pre-Analytical Stability. *Clin*
3 *Chem Lab Med* 2003;41:700–3.
- 4 16. Djukanovic D, Hofmann U, Sucker A, Schadendorf D. Melanoma tumour markers S100B
5 and MIA: evaluation of stability in serum and blood upon storage and processing. *Br J*
6 *Dermatol* 2001;145:1030–1.
- 7 17. Ferial J, Adamo F, Monneret D, Trehel-Tursis V, Favard S, Tsé C, et al. S100B protein
8 concentration measurement according to two different immunoassays. *Clin Chem Lab Med*
9 *CCLM* 2015;53:e169–71.
- 10 18. Delefortrie Q, Lejeune F, Kerzmann B, Levy R, Adam J-F, Sottiaux T, et al. Evaluation of
11 the Roche® Elecsys and the Diasorin® Liaison S100 kits in the management of mild head
12 injury in the emergency room. *Clin Biochem* 2018;52:123–30.
- 13 19. Biberthaler P, Linsenmeier U, Pfeifer K-J, Kroetz M, Mussack T, Kanz K-G, et al. Serum
14 S-100B concentration provides additional information for the indication of computed
15 tomography in patients after minor head injury: a prospective multicenter study. *Shock*
16 *Augusta* 2006;25:446–53.
- 17 20. Mercier E, Boutin A, Lauzier F, Fergusson DA, Simard J-F, Zarychanski R, et al.
18 Predictive value of S-100 β protein for prognosis in patients with moderate and severe
19 traumatic brain injury: systematic review and meta-analysis. *BMJ* 2013;346:f1757.
- 20 21. Allouchery G, Moustafa F, Roubin J, Pereira B, Schmidt J, Raconnat J, et al. Clinical
21 validation of S100B in the management of a mild traumatic brain injury: issues from an
22 interventional cohort of 1449 adult patients. *Clin Chem Lab Med* 2018;56:1897–1904.
- 23 22. Mazer CD, Novoshinov S, Tsoporis J, Izhar S, Tousignant C, Latter D, et al. Heparin
24 influences s100b protein levels in blood during cardiac surgery. *Can J Anesth*
25 2007;54:44607–44607.
- 26 23. Wang S, Wang L, Zhang X, Wang C, Zheng G, Wu W, et al. Effects of subcutaneous low
27 molecular weight heparin and intravenous unfractionated heparin on serum S100
28 concentrations in patients with cerebrovascular diseases. *Clin Chem Lab Med* 2012;50:525–8.
- 29 24. Steiner J, Marquardt N, Pauls I, Schiltz K, Rahmoune H, Bahn S, et al. Human CD8(+) T
30 cells and NK cells express and secrete S100B upon stimulation. *Brain Behav Immun*
31 2011;25:1233–41.
- 32 25. Padoan A, Zaninotto M, Piva E, Sciacovelli L, Aita A, Tasinato A, et al. Quality of
33 plasma samples and BD Vacutainer Barricor tubes: Effects of centrifugation. *Clin Chim Acta*
34 2018;483:271–4.
- 35 26. Bouvier D, Castellani C, Fournier M, Dauphin J-B, Ughetto S, Breton M, et al. Reference
36 ranges for serum S100B protein during the first three years of life. *Clin Biochem*
37 2011;44:927–

38

39

1 **Figure 1** Pre-analytical study: matrix effect and stability. **(A)** Storage at room temperature
2 and at 2–8°C. **(B)** Storage at -20°C and -80°C. Tube a: serum separator tube with gel; tube b:
3 serum separator tube without gel; tube c: lithium heparinate gel separator tube; T0:
4 measurement after centrifugation; T24: measurement after storage for 24 h (8 h at room
5 temperature and 16 h at 2–8°C); T48: measurement after storage for 48h (8 h at room
6 temperature and 40 h at 2–8°C).

7

8 **Figure 2** Plot of the differences in the S100B concentrations between the serum and plasma
9 samples against the average of the two samples. Tube a: serum gel separator tubes; Tube c:
10 lithium heparinate gel separator tubes; SD: standard deviation.

11

12 **Table 1** Summary of patient demographics. GCS: Glasgow Coma Scale; min: minimum;
13 max: maximum; IQR: interquartile range; TBI: Traumatic Brain Injury.

14

15 **Table 2** Stability of the S100B protein after storage for 48 h (8 h at room temperature and 40
16 h at 2–8°C). [S100B]: S100B concentration (µg/L); min: minimum; max: maximum; IQR:
17 interquartile range; tube a: serum separator tube with gel; tube b: serum separator tube
18 without gel; tube c: lithium heparinate gel separator tube; T0: measurement after
19 centrifugation; T24: measurement after storage for 24 h (8 h at room temperature and 16 h at
20 2–8°C); T48: measurement after storage for 48h (8 h at room temperature and 40 h at 2–8°C);
21 *p*: a *p* value < 0.05 was considered statistically significant.

22

23 **Table 3** Stability of S100B protein after storage for 6 months at -20°C. [S100B]: S100B
24 concentration (µg/L); min: minimum; max: maximum; IQR: interquartile range; tube a: serum
25 separator tube with gel; tube c: lithium heparinate gel separator tube; T0: measurement after
26 centrifugation; *p*: a *p* value <0.05 was considered to be statistically significant.

27

28 **Table 4** Stability of S100B protein after storage for 9 months at -80°C. [S100B]: S100B
29 concentration (µg/L); min: minimum; max: maximum; IQR: interquartile range; tube a: serum
30 separator tube with gel; tube c: lithium heparinate gel separator tube; T0: measurement after
31 centrifugation; *p*: a *p* value <0.05 was considered to be statistically significant.

32

33 **Table 5** Precision study of the S100B prototype assay on a bioMérieux Vidas[®] 3 analyzer.
34 CV: coefficient of variation; L: low; H: high; SD: standard deviation.

1
2
3
4
5
6
7
8
9
10
11
12
13

Figure 3 Correlation between the bioMérieux Vidas[®] 3 prototype and the Roche Diagnostics Cobas e411[®] for S100B concentrations. **(A)** Linear regression. **(B)** Bland-Altman plot; SD: standard deviation.

Table 6 Comparison of the clinical performance between the two S100B assays: Roche Diagnostics Cobas e411[®] and bioMérieux Vidas[®] 3 prototype. CT scan-: CT scan without intracerebral lesion; CT scan+: CT scan with intracerebral lesion; S100B-: S100B concentration <0.10 µg/L; S100B+: S100B concentration ≥0.10 µg/L.

1 **Table 1**
2

| | Neuro-Critical Care Unit (n = 71) | Emergency Department (n = 80) |
|--|---|--|
| Age median, years (min; max; IQR) | 60 (20; 87; 49–69) | 42 (18; 91; 21–65) |
| Sex-ratio: male/female | 1.34 | 1.58 |
| GCS median (min; max; IQR) | 7 (3; 15; 5–12) | 15 (14; 15; 15–15) |
| Clinical contexts | <ul style="list-style-type: none"> - Subarachnoid hemorrhage (n = 18) - Neurosurgical postoperative (n = 19) - Moderate TBI (n = 8) - Severe TBI (n = 7) - Intraparenchymal hemorrhage (n = 8) - Ischemic stroke (n = 5) - Brain tumor (n = 3) - Mild TBI (n = 2) - Status epilepticus (n = 1) | Mild TBI (n = 80) |
| Usefulness of samples | <ul style="list-style-type: none"> - Matrix effect and storage stability - Precision and comparison of the methods | <ul style="list-style-type: none"> - Comparison of the methods - Clinical diagnostics performance in context of mTBI |

3

1 **Table 2**

2

| Tubes | Median [S100B] (min; max; IQR) | | | <i>p</i> |
|----------|-----------------------------------|---------------------------------|---------------------------------|-------------|
| | T0 | T24 | T48 | |
| a | 0.20 (0.03; 4.37; 0.08–0.56) | 0.20 (0.04; 4.35; 0.08–0.50) | 0.17 (0.04; 4.13; 0.07–0.43) | 0.83 |
| b | 0.20 (0.03; 4.59; 0.08–0.54) | 0.17 (0.04; 4.37; 0.08–0.48) | 0.18 (0.04; 4.23; 0.07–0.43) | 0.95 |
| c | 0.23 (0.05; 4.77; 0.10–0.61) | 0.22 (0.05; 4.50; 0.09–0.54) | 0.19 (0.04; 4.08; 0.08–0.48) | 0.56 |

3

4

5

6

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

28

29

30

31

32

33

34

35

36

37

38

39

40

1
2 **Table 3**

| Tubes | Median [S100B] (min; max; IQR) | | | | <i>p</i> |
|----------|-----------------------------------|---------------------------------|---------------------------------|---------------------------------|-------------|
| | T0 | 1 month at -20°C | 3 months at -20°C | 6 months at -20°C | |
| a | 0.22 (0.03; 5.27; 0.09–0.65) | 0.23 (0.04; 5.38; 0.09–0.60) | 0.22 (0.03; 5.04; 0.09–0.54) | 0.22 (0.03; 5.19; 0.08–0.60) | 0.92 |
| c | 0.26 (0.05; 5.58; 0.11–0.64) | 0.29 (0.05; 5.58; 0.12–0.68) | 0.26 (0.03; 5.15; 0.11–0.57) | 0.27 (0.04; 5.43; 0.10–0.65) | 0.82 |

3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36

1

| Tubes | Median [S100B] (min; max; IQR) | | | <i>p</i> |
|----------|-----------------------------------|---------------------------------|---------------------------------|-------------|
| | T0 | 3 months at -80°C | 9 months at -80°C | |
| a | 0.22 (0.03; 5.27; 0.09–0.65) | 0.22 (0.03; 5.05; 0.09–0.55) | 0.22 (0.04; 5.36; 0.09–0.61) | 0.97 |
| c | 0.26 (0.05; 5.58; 0.11–0.64) | 0.30 (0.04; 5.07; 0.11–0.66) | 0.31 (0.04; 5.58; 0.11–0.68) | 0.93 |

2

3 **Table 4**

4

5

6

7

8

9

10

11

12

13

14

15

16

17

18

19

20

21

22

23

24

25

26

27

1

2

3

4

5 **Table 5**

| Samples | | Repeatability | | | | Reproducibility | | | |
|--------------|----|---------------|------------|------------|----------------------|-----------------|------------|------------|----------------------|
| | N | Mean µg/L | SD µg/L | CV % | Manufacturer CV % | Mean µg/L | SD µg/L | CV % | Manufacturer CV % |
| L pool serum | 30 | 0.07 | 0.001 | 1.7 | 2.9 | 0.07 | 0.003 | 3.9 | 3.9 |
| L control | 30 | 0.09 | 0.002 | 2.0 | 2.6 | 0.09 | 0.003 | 3.1 | 5.6 |
| H pool serum | 30 | 0.66 | 0.013 | 2.0 | 3.4 | 0.66 | 0.021 | 3.2 | 3.5 |
| H control | 30 | 0.42 | 0.009 | 2.2 | 2.4 | 0.42 | 0.012 | 2.8 | 3.5 |

6

7

8

1 **Table 6**

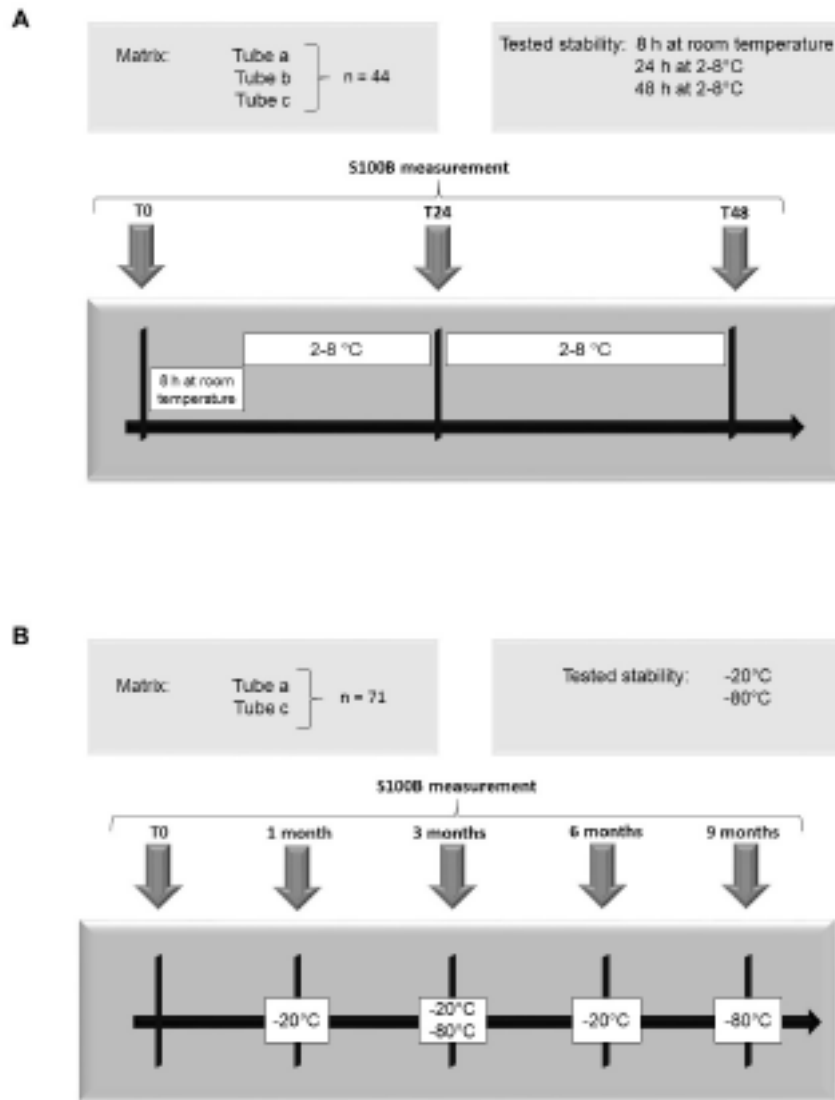
2
3
4

| | | COBAS e411[®] n (%) | Vidas[®] 3 n (%) | <i>p</i> |
|-------------------------------------|----------|---|--|-------------|
| S100B - | | 32 (40) | 30 (37.5) | 0.41 |
| No CT scan prescription | | 28 (35) | 24 (30) | 0.50 |
| CT scan prescription (all CT scans) | | 4 (5) | 6 (7.5) | 0.50 |
| S100B + | | 48 (60) | 50 (62.5) | 0.41 |
| No CT scan prescription | | 4 (5) | 8 (10) | 0.53 |
| CT scan prescription | CT scan+ | 3 (3.7) | 3 (3.7) | 0.53 |
| | CT scan- | 41 (51.3) | 39 (48.8) | 0.53 |

5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24

1

2 **Figure 1**



3

4

5

6

7

8

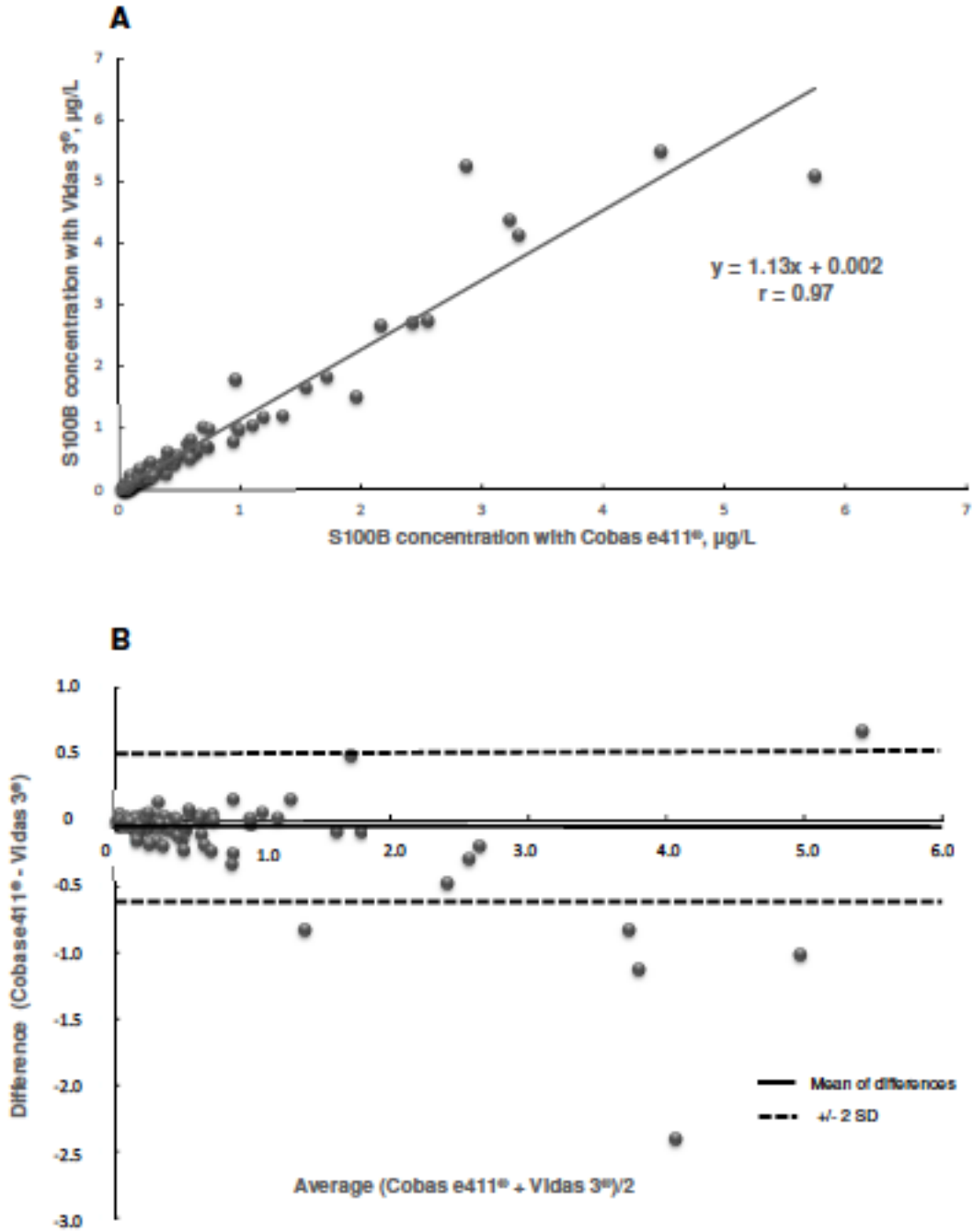
9

10

11

1
2
3

Figure 3



4
5