S100B Protein in Clinical Diagnostics: Assay Specificity, Claus W. Heizmann (Division of Clinical Chemistry & Biochemistry, Department of Pediatrics, University of Zurich, Steinwiesstrasse 75, CH-8032 Zurich, Switzerland; fax 41-1-266-7169, e-mail Claus.Heizmann@kispi.unizh.ch)

S100 proteins/antibodies have received increasing attention because of their use in the diagnosis of several human diseases and as predictive markers of improving clinical management, outcome, and survival of patients (1, 2).

S100 proteins also have a great potential as drug targets to improve therapies. Unique to the S100 family is the location of 16 S100 genes in a gene cluster on human chromosome 1q21; this led to the introduction of the widely accepted nomenclature shown in Table 1. Four additional S100 genes, including S100B, are located on different chromosomes [for reviews, see Refs. (1, 3); for S100A15, see Ref. (4); for S100A16, see I. Marenholz and C.W. Heizmann, Characterization of S100A16, an ubiquitously expressed EF-hand protein which is upregulated in tumors, submitted for publication].

S100 proteins are 10–12 kDa in size and form homo- and heterodimers. The monomer is composed of two helix-loop-helix (EF-hand) motifs connected by a central hinge region (1, 3). The C-terminal EF-hand contains the canonical Ca$^{2+}$-binding loop, common to all EF-hand proteins (e.g., troponin C or calmodulin). The N-terminal EF-hand consists of 14 amino acids and is characteristic of S100 proteins. S100 proteins show different degrees of homology, ranging from 25% to 65% identity at the amino acid level.

Generally, the dimeric S100 proteins bind four Ca$^{2+}$ per dimer ($K_d = 20–500$ µmol/L). In addition to Ca$^{2+}$, several S100 proteins bind Zn$^{2+}$ with a wide range of affinities ($K_d = 0.1–2000$ µmol/L), and some (S100B and S100A5) even

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References

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Fig. 1. PCR-RFLP (A) and direct sequencing results (B and C) for the CYP3A5 allele.

(A), PCR-RFLP results for CYP3A5*3. The PCR products were analyzed on a 3% agarose gel with a 50-bp DNA marker. Lane 0, uncut PCR product; lane 1, homozygous *3/*3 individual; lane 2, heterozygous *1/*3 individual; lane 3, wild-type *1/*1 individual. (B and C), direct sequencing results for products obtained with primers P1 and P2 (B) and with primers P3 and P4 (C) for a homozygous *3/*3 individual (panel 1), a heterozygous *1/*3 individual (panel 2), and a wild-type *1/*1 individual (panel 3).
bind Cu$^{2+}$ ($K_d = 0.4–5 \mu\text{mol/L}$). This suggests that S100 protein–target interactions and cellular functions may be triggered by Ca$^{2+}$, Zn$^{2+}$, and Cu$^{2+}$ (5).

The individual members of the S100 protein family show a tissue- and cell-type-specific expression pattern; for example, there are high concentrations of S100B and S100A6 in the brain (6–8). Deregulation of S100 gene expression is observed in several human diseases, which has made individual S100 proteins valuable diagnostic markers for conditions such as inflammation and wound healing (S100A8, -A9, and -A12), cardiomyopathy/myocardial infarction (S100A1), classification of human tumors (S100A2, -A4, and -A6), and in evaluating the metastatic potential/prognosis of patients (S100A2 and -A4).

Furthermore, some S100 proteins (including S100B) are even secreted from cells exhibiting extracellular, cytokine-like activities partially via the surface receptor for advanced glycation end products (9, 10) with paracrine effects on neurons. The extracellular concentrations of S100 proteins play a crucial role in their physiologic response. For example, nanomolar concentrations of S100B have trophic effects on cells, but the pathologic concentrations found in Alzheimer patients lead to glial activation and apoptosis (11). Thus, S100 protein concentrations measured in body fluids may be composed of S100 proteins released from damaged cells as well as S100 proteins secreted under pathologic conditions.

S100B was the first member of the S100 protein family to be measured in various body fluids to test its usefulness as index of brain damage in children and adults [for a review, see Ref. (2)]. S100B concentrations correlated well with the extent of brain damage occurring in neurodegenerative disorders, traumatic or focal insults, or during open heart surgery. S100B was also suggested to be a noninvasive marker for blood–brain barrier function (12).

Finally, S100B was increased in the blood of patients with malignant melanoma; melanocytes are known to express high amounts of this protein (13–15).

S100B concentrations in body fluids are usually determined by commercially available immunoassays such as the Can Ag S100 EIA (Can Ag Diagnostics AB, Gothenburg, Sweden), the ELISA NEXUS DXTM S100 (Syn X Pharma Inc., York, U.K.), or the widely used two-site immunoassays (Sangtec® 100 IRMA, Liaison® Sangtec 100, Sangtec 100 ELISA) from DiaSorin AB (Bromma, Sweden).

We have tested the specificity of the Sangtec 100 IRMA assay for S100B. At the time when this assay was developed, only two S100 proteins (S100B and S100A1) were well known; therefore, a test for possible cross-reactivity with the other homologous members of this protein family exhibiting similar structural features was not possible.

The Sangtec immunoassay is based on three monoclonal antibodies raised against three peptides derived from bovine S100B, described previously (16). Therefore, the first task was to test whether these monoclonal antibodies also recognized human recombinant S100B. The second goal was to test the specificity of this S100B assay against several other S100 proteins. In a first step we cloned human S100B, S100A1 through -A6, S100A12, and S100A13 into expression vectors for high expression in Escherichia coli and purified the recombinant proteins by ammonium sulfate precipitation, followed by Ca$^{2+}$-de-
ependent hydrophobic interaction, size-exclusion, and/or exchange chromatography (1, 3).

Purified dimeric S100 proteins were characterized by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and their metal-binding and immunologic properties. The exact masses of S100 proteins were determined by electrospray ionization mass spectrometry (SCIEX API 365; Perkin-Elmer). Native proteins (in contrast to the recombinant proteins) were found to be mostly acetylated with no other posttranslational modifications. Human S100A8 and S100A9 were purchased from Bachem AG.

The protocol of the supplier was followed except that the Sangtec 100 IRMA calibrators (containing lyophilized partly purified bovine S100 peptides) were replaced by the same concentration (0.5–60 μg/L) of human recombinant S100 proteins. The reagent blank (negative control) contained no protein calibrators. As illustrated in Fig. 1, the assay recognized the human S100B protein equally well and in the same concentration range as the bovine S100B calibrator.

The Sangtec IRMA was linear over a range of 0–20 μg/L; the same linearity was obtained with identical concentrations of the recombinant human S100B.

To test the specificity of this assay, we replaced the bovine S100B calibrator with the various human recombinant S100 proteins in concentrations of 10–30 μg/L. There was no interference/cross-reactivity by the other S100 proteins tested, including S100A6 and S100A4, which are highly expressed in brain tissue.

According to these results this diagnostic assay is specific and reliable for measurement of S100B in human body fluids.

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References

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Multicenter Characterization and Validation of the Intron-8 Poly(T) Tract (IVS8-T) Status in 25 Coriell Cell Repository Cystic Fibrosis Reference Cell Lines for Cystic Fibrosis Transmembrane Conductance Regulator (CFTR) Gene Mutation Assays, Siby Sebastian, Silvia G. Spitzer, Leonard E. Grosso, Jean Amos, Frederick V. Schaefer, Elaine Lyon, Daynna J. Wolff, Atieh Hajianpour, Annette K. Taylor, Alison Millson, and Timothy T. Stenzel (1 Department of Pathology, Molecular Diagnostics Laboratory, Duke University Medical Center, Durham, NC; 2 Molecular Genetics Laboratory of SUNY at Stony Brook, Stony Brook, NY; 3 Department of Pathology, St. Louis University School of Medicine, St. Louis, MO; 4 Specialty Laboratories Inc., Santa Monica, CA; 5 Chapman Institute of Medical Genetics, Tulsa, OK; 6 ARUP Laboratories, University of Utah, Salt Lake City, UT; 7 Department of Pathology and Laboratory Medicine, Medical University