Biotin Interference in Clinical Immunoassays: A Cause for Concern

To the Editor.—An increase in the use of biotin supplements by the general public is producing an increase in the number of reports of analytic interference in biotin-based immunoassays (BBAs) used to evaluate endocrine function.[1,2] The fact that BBAs of similar design are currently used to diagnose and manage a wide range of other medical conditions, including anemias, malignancies, autoimmune and infectious diseases, and cardiac damage, raises the concern that the accuracy of results for other routine tests are compromised as well.[3] Methods that use immunometric ("sandwich") or competitive formats are at the greatest risk for producing falsely decreased or falsely increased results, respectively, when biotin is present in the sample.

In June of 2016, we reviewed the current manufacturers' instructions for use for 374 methods performed by 8 of the most popular immunoassay analyzers used in the United States. Two hundred twenty-one of the methods were BBAs. Eighty-two of these were immunometric or competitive methods that had manufacturer-reported interference thresholds (IFTs) (ie, the concentrations above which exogenous biotin in the sample caused a difference of greater than ±10% in the test result) of less than 51 ng/mL. Another 15 of the methods neither reported an IFT nor identified biotin as a potential interfering substance (Table).

Healthy subjects who take a single 1-mg or 100-mg oral dose of biotin have mean peak serum biotin concentrations of 8.6[4] and 495[5] ng/mL, respectively, occurring between 1 and 3 hours post dose and declining with half-lives of 8 to 19 hours. The peak serum biotin concentration following a 1-mg oral dose would, therefore, be expected to increase the risk of an erroneous test result measured by a vulnerable method with an interference threshold of less than 8.6 ng/mL. While peak serum biotin concentrations after oral doses of 5 and 10 mg—the doses most frequently used by the public—have yet to be reported, the 100% bioavailability of such doses[5] predicts that they would be even more likely to affect the accuracy of immunoassays with low interference thresholds. And certainly, the peak biotin concentration following a single 100-mg dose would be likely to decrease the analytic accuracy of all 82 of the methods that have thresholds of less than 51 ng/mL. In this case, the risk of an erroneous test result could persist for up to 8 elimination half-lives (>6 days) for a method with an IFT of 2.4 ng/mL. Therefore, it might be necessary to avoid the use of some BBAs altogether when testing subjects who

Biotin-Based Immunoassays at High Risk for Analytic Interference by Biotin Supplements

Multitest Assay System	No. of Methods			Vulnerable Immunometric and Competitive Methods With IFTs of <51 ng/mL or no IFTs Reported in the Product Labeling
	Total	BBAs	High Risk[a]	Method(s) [IFT in ng/mL] or Method(s) [NR][b]
Elecsys[c]	66	66	44	Folate **[5]**; anti-HBsAg **[8]**; anti-TPO, anti-TSHR, and total T3 **[10]**; free T4, progesterone, and TnT **[20]**; TSH **[25]**; anti-CCP, anti-HBc, procalcitonon, CK-MB, cortisol, DHEAS, free PSA, GH, BNP, TSTN, thyroglobulin, and TnI **[30]**; CA 125 **[35]**; estradiol **[36]**; calcitonin, HBeAg, HBsAg, hCG, prolactin, and T uptake **[40]**; anti-HCV **[42]**; iPTH, anti-HAV, anti-HAV IgM, B12, Cyfra 21-1, ferritin, HE4, HSV-1 IgG, HSV-2 IgG, LH, myoglobin, osteocalcin, rubella IgG, and rubella IgM **[50]**
Vitros[d]	37	30	28	TnI **[2.4]**; estradiol, iPTH, LH, and TSH **[4.8]**; cortisol, hCG, AFP, anti-HAV, anti-HAV IgM, anti-HBe, CA 125, CA 15-3, CEA, CK-MB, ferritin, folate, FSH, HBeAg, prolactin, TSTN, and total PSA **[10]**; 25 OHD **[15]**; B12, myoglobin, BNP, and progesterone **[20]**; anti-HBc IgM **[NR]**
Access/DXI[e]	37	15	6	Free T3 **[10]**; CA 19-9, free T4, myoglobin, thyroglobulin, and total T3 **[NR]**
Centaur[f]	65	23	7	HBsAG and TnI **[10]**; folate **[13]**; HAV total **[25]**; TSTN and anti-HBc IgM **[30]**; anti-HAV IgM **[50]**
Immulite 2000[f]	60	60	6	IgE allergy, gastrin, CA 15-3, CRP, free T3, and thyroglobulin **[NR]**
Dimension[f]	26	23	6	Free T3, free T4, and digoxin **[50]**; hCG, myoglobin, and TnI **[NR]**
Architect i2000[g]	47	4	0	None
Liaison XL[h]	36	0	0	None

Abbreviations: 25 OHD, 25 hydroxyvitamin D; AFP, α-fetoprotein; anti-, antibody to; B12, vitamin B12; BBA, biotin-based immunoassay; BNP, brain natriuretic peptide; CA 125, cancer antigen 125; CA 15-3, cancer antigen 15-3; CA 19-9, cancer antigen 19-9; CCP, cyclic citrullinated peptide; CEA, carcinoembryonic antigen; CK-MB, creatine kinase MB isoenzyme; CRP, C-reactive protein; Cyfra 21-1, fragments of cytokeratin-19; DHEAS, dehydroepiandrosterone sulfate; FSH, follicle-stimulating hormone; GH, growth hormone; HAV, hepatitis A virus; HBc, hepatitis B core antigen; HBeAg, hepatitis Be antigen; HBsAg, hepatitis B surface antigen; hCG, human chorionic gonadotropin; HCV, hepatitis C virus; HE4, human epididymis secretory protein 4; HSV-1 IgG, IgG antibodies to herpes simplex virus, type 1; HSV-2 IgG, IgG antibodies to herpes simplex virus, type 2; IFT, interference threshold; IgE, immunoglobulin E; IgG, immunoglobulin G; IgM, immunoglobulin M; iPTH, intact parathyroid hormone; LH, luteinizing hormone; NR, not reported; PSA, prostate-specific antigen; T3, triiodothyronine; T4, thyroxine; TnI, troponin I; TnT, troponin T; TPO, thyroid peroxidase; TSH, thyroid-stimulating hormone; TSHR, thyrotropin receptor; TSTN, testosterone.

[a] Immunometric and competitive assay formats in which biotinylated reagents bind to streptavidin or anti-biotin antibody reagents in the presence of exogenous biotin in the patient sample.
[b] Neither an IFT nor a statement acknowledging the potential for analytic interference by biotin was reported in the product labeling.
[c] Roche Diagnostics, Indianapolis, Indiana.
[d] Ortho Clinical Diagnostics Inc, Rochester, New York.
[e] Beckman Coulter Inc, Fullerton, California.
[f] Siemens Healthcare Diagnostics Inc, Tarrytown, New York.
[g] Abbott Laboratories, Abbott Park, Illinois.
[h] DiaSorin Inc, Stillwater, Minnesota.

are using the 100-mg biotin supplements that are now available over-the-counter.[2,5]

The confluence of increased biotin supplement use by patients and the design limitations of many of the BBAs have already led to the misdiagnosis and mismanagement of patients.[1–3] We are convinced that the risk of analytic interference by biotin supplements is a serious problem that needs to be more widely recognized and promptly addressed by health care providers, directors of clinical laboratories, and decision makers in the clinical diagnostics industry.

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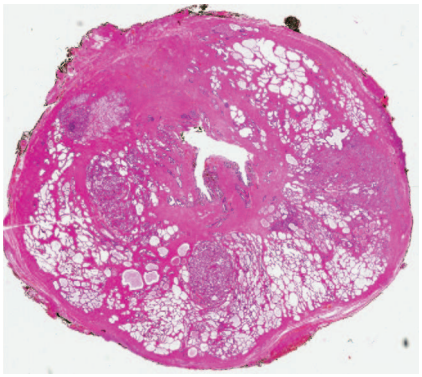
Whole Slide Imaging of Large Format Histology in Prostate Pathology: Potential for Information Fusion

To the Editor.—We read with considerable interest the contribution written by Alton Brad Farris, MD, and colleagues[1] in the April 2017 issue of the *Archives of Pathology & Laboratory Medicine*, entitled "Whole Slide Imaging for Analytical Anatomic Pathology and Telepathology: Practical Applications Today, Promises, and Perils." They have led us, a transnational group of closely collaborating pathologists, urologists, oncologists, engineers, and informaticians, to some considerations on our past and current role in this new era of digitalization of glass slides, that is, whole slide imaging (WSI) of genitourinary neoplasms, in particular, of prostate pathology.[2,3]

This new era of WSI requires knowledge of previous studies that contributed to the current use and role of virtual slides and quantitative tissue analysis, for instance, in prostate cancer detection and grading, as well as in characterization of high-grade prostatic intraepithelial neoplasia and malignancy-associated changes.[2] In the past 20 to 30 years, the technologic advancements have reached the point that we can obtain a virtual slide in the range of megabytes to gigabytes accessible, even in tablets and cell phones, for sharing, as well as for joint evaluation in a multidisciplinary setting, including quantitative image analysis.[2] Evaluation of prostate histology on virtual slides can offer clues to the diagnostic classification and prognosis and the prediction of response to treatment. To facilitate the collection of quantitative data, machine-vision systems have been developed and Bayesian belief networks and neural networks have been used as diagnostic decision-support systems.[2] All these approaches can be seen as the basis for simultaneous, quantitative evaluation of several tissue markers, including immunohistochemical and molecular patterns, in a multiplex system.[2]

There are several advantages currently associated with the digitalization of glass slides in this new era of WSI, including image sharing for teaching, consultation, remote interpretation, and quality assurance.[4,5] Additional features are "interactive publication" (similar to online, scientific chats) and image analysis (readers might use measurement systems available in the Web).[6] The whole point of a journal article based on WSI is education, that is, the transfer of knowledge, experience, and guidance.[7] In our experience, WSI forms an ideal basis for sound communication and represents a major component in medical diagnosis and treatment.



Example of large format histology: whole mount hematoxylin-eosin–stained section of a radical prostatectomy specimen. For information on accessing the whole slide image, please contact the author at r.montironi@univpm.it (original magnification ×1).

There are 2 basic types of glass slides, based on their size and, therefore, of virtual slides derived from them. The most common is the glass slide from material processed in a regular tissue cassette (dimensions, 30 × 25 × 4 mm). The other, far less common, is the glass slide from material processed with a large tissue cassette or megacassette (dimensions, 63 × 47 × 11 mm). The latter is also called large format histology (LFH) or whole mount sections.[8] We have applied LFH to basically all types of surgical specimens of bladder, testis, kidney, adrenal gland, penis, and prostate, including their lymphadenectomy. Virtual slides can be obtained from both types of glass slides. The WSI is traditionally based on slides from regular tissue cassettes. In the past few years, we have been able to scan several whole mount sections with a commercially available slide scanner (Figure). This has allowed us to acquire a unique experience in the joint evaluation with clinicians of virtual slides from large-format histology.[9,10] Our experience of LFH and WSI is basically related to the field of uropathology. However, it can be performed in all body organs and neoplasms, including breast and its cancers,[11] in which LFH can be adopted. To our knowledge, not all commercially available slide scanners allow the users to obtain virtual slides from whole mount sections. However, because of advantages with LFH and