Our results show that inserting an indexing spot in the microarray is an effective approach to alert for heterophilic antibody interference in antibody microarray–based immunoassays; however, results obtained with the unrelated monoclonal antibodies of the index spot may not be totally the same as the capture antibodies in an antibody array. As more samples are tested, samples with confusing results that cannot be interpreted with the MMAB index may also be found. Thus, there remains a need to improve and explore identification methods for such samples.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, acquisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.*

Authors' Disclosures or Potential Conflicts of Interest: *No authors declared any potential conflicts of interest.*

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

References

1. Ellington AA, Kullo IJ, Bailey KR, Klee GG. Antibody-based protein multiplex platforms: technical and operational challenges. Clin Chem 2010;56:186–93.
2. Boscato LM, Stuart MC. Heterophilic antibodies: a problem for all immunoassays. Clin Chem 1988;34:27–33.
3. Kuroki M, Matsumoto Y, Arakawa F, Haruno M, Murakami M, Kuwahara M, et al. Reducing interference from heterophilic antibodies in a two-site immunoassay for carcinoembryonic antigen (CEA) by using a human/mouse chimeric antibody to CEA as the tracer. J Immunol Methods 1995;180:81–91.
4. Butler SA, Cole LA. Use of heterophilic antibody blocking agent (HBT) in reducing false-positive hCG results. Clin Chem 2001;47:1332–3.
5. Levit-Binnun N, Lindner AB, Zik O, Eshhar Z, Moses E. Quantitative detection of protein arrays. Anal Chem 2003;75:1436–41.

Xiaoli Yang[2,3]
Haipeng Ji[3]
Xiaolin Lin[3]
Zhijun Han[2]
Feizhou Liu[3*]
Renqian Zhong[2*]

[2] *Changzheng Hospital and*
[3] *Shanghai HealthDigit Co. Ltd.*
Shanghai, P.R. China

* Address correspondence to:
F. Liu at
Shanghai HealthDigit Co. Ltd.
699 North Huifeng Rd.
201403 Shanghai, P.R. China
E-mail liufeizhou@health-digit.com
R. Zhong at
Changzheng Hospital
415 Fengyang Rd.
200002 Shanghai, P.R. China
E-mail rqzhong@yahoo.com

Previously published online at
DOI: 10.1373/clinchem.2010.156448

Plasma Renin Activity Enzyme-Kinetic Assay: Protection of Angiotensin I from Bacterial Degradation

To the Editor:

Bystrom et al. *(1)* recently reported substantial peptidase activity in human plasma that had been incubated at 37 °C to generate angiotensin I (Ang I)[1] during the clinical plasma renin activity (PRA) assay. If this result is correct, it would call into question many of the PRA results previously reported by our laboratory and by Quest Diagnostics, especially those with low PRA ($<0.65\ \mu g \cdot L^{-} \cdot h^{-}$). We believe, however, this observation is most likely an artifact caused by a failure to follow published protocols *(2, 3)*.

[1] Nonstandard abbreviations: Ang I, angiotensin I; PRA, plasma renin activity.

Several features of the Ang I degradation reported by Bystrom et al. *(1)* indicate the possibility of bacterial contamination: (*a*) the presence of both carboxypeptidase and aminopeptidase-like activity (their Fig. 4C), (*b*) a lower rate of Ang I degradation during the shorter Ang I–generation times (their Fig. 2), (*c*) the independence from the PRA, and (*d*) the lack of any reported indication of specificity by patient or blood-collection site. Bacterial contamination could occur at many sites: from the patient, at the blood-collection site, during separation of the plasma, and in the laboratory, to name a few.

Given that plasma is an excellent incubation medium for bacteria, we have long considered bacterial contamination a potential source of angiotensin peptide degradation during the Ang I–generation step of the clinical PRA assay *(2, 3)*. For that reason, we routinely add neomycin sulfate to plasma samples before carrying out the Ang I–generation step at 37 °C. Bystrom et al. did not do that. Adding neomycin sulfate is like an insurance policy: Most of the time it is not necessary, but sometimes it is essential. We suggest that Bystrom et al. directly test whether adherence to the classic protocol, including adding neomycin, succeeds in reducing or eliminating the problem of sporadic degradation of Ang I observed in their assay. Until then, it would be prudent to restore adding a bactericidal agent to the Ang I–generation step of their clinical PRA assay or to use another means to protect the generated Ang I, such as anti–Ang I antibodies *(4)* or heat inactivation of protein-containing assay buffers.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant contributions to the conception and design, ac-*

quisition of data, or analysis and interpretation of data; (b) drafting or revising the article for intellectual content; and (c) final approval of the published article.

Authors' Disclosures or Potential Conflicts of Interest: *Upon manuscript submission, all authors completed the Disclosures of Potential Conflict of Interest form. Potential conflicts of interest:*

Employment or Leadership: J.E. Sealey, *American Journal of Hypertension*; J.H. Laragh, *American Journal of Hypertension*.
Consultant or Advisory Role: J.E. Sealey, Diasorin; J.H. Laragh, Diasorin.
Stock Ownership: None declared.
Honoraria: None declared.
Research Funding: None declared.
Expert Testimony: None declared.

Role of Sponsor: The funding organizations played no role in the design of study, choice of enrolled patients, review and interpretation of data, or preparation or approval of manuscript.

References

1. Bystrom CE, Salameh W, Reitz R, Clarke NJ. Plasma renin activity by LC-MS/MS: development of a prototypical clinical assay reveals a subpopulation of human plasma samples with substantial peptidase activity. Clin Chem 2010; 56:1561–9.
2. Sealey JE, Laragh JH. Radioimmunoassay of plasma renin activity. Semin Nucl Med 1975;5:189–202.
3. Sealey JE. Plasma renin activity and plasma prorenin assays. Clin Chem 1991;37:1811–9.
4. Nussberger J, Fasanella d'Amore T, Porchet M, Waeber B, Brunner DB, et al. Repeated administration of the converting enzyme inhibitor cilazapril to normal volunteers. J Cardiovasc Pharmacol 1987;9:39–44.

Jean E. Sealey[2,3*]
John H. Laragh[3]

Departments of [2] Medicine and [3] Cardiothoracic Surgery
Weill Cornell Medical College
New York, NY

* Address correspondence to this author at:
Weill Cornell Medical College
1300 York Ave.
Box 266
New York, NY 10065-4805
Fax 561-369-3479
E-mail: jsealey@med.cornell.edu

Previously published online at
DOI: 10.1373/clinchem.2010.156596

In Reply

We thank Drs. Sealy and Laragh for their thoughts on the nature of the degradation activity against angiotensin I (Ang I) described in our recent report.

During development of the assay, we identified a number of samples in which the 18-h RIA results generated with the Sealey/Laragh method were below the limit of quantification but the liquid chromatography–tandem mass spectrometry (LC-MS/MS) method produced results in the range of 0.3–0.7 $\mu g \cdot L^{-1} \cdot h^{-1}$. In these experiments, neomycin sulfate was used in the RIA but not in the Ang I–generation mixture used for LC-MS/MS. These discordant results led us to explore the nature of the degradation phenomena. Because of the concerns raised, we have repeated our experiments and have again examined the difference in our current method with and without the addition of neomycin. A collection of split patient samples that included both the typical (n = 50) and high-degradation phenotypes (n = 23) were tested in our commercial assay with and without the addition of neomycin. We found no statistically significant difference in the loss of the degradation indicator (P = 0.12, pairwise t-test; Fig. 1). In samples with the high-degradation phenotype, Ang I was below the limit of quantification in all but 2 samples. For typical samples, the correlation of the plasma renin activity results for the samples processed with and without neomycin was excellent (m = 1.004; b = 0.02; r^2 = 0.954).

Profound degradation activity was observed in some plasma samples, even during very short incubation times (≤15 min), and these observations appear incompatible with bacterial growth during Ang I generation. In the case of our plasma renin activity assay, blood samples are carefully collected into sterile Vacutainers, and the plasma fraction is frozen immediately after separation. Our practice of only thawing immediately before analysis provides little opportunity for bacterial growth before the generation step. Even if bacterial contamination were to occur before generation, the presence of neomycin would serve only to prevent additional bacterial growth; it would not address the strong degradation activity that we monitored with the degradation calibrator.

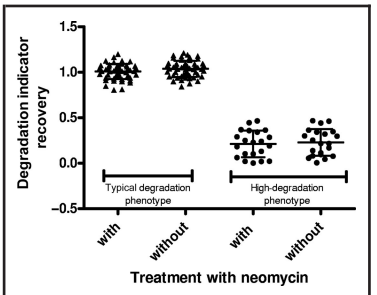

Fig. 1. Comparison of recoveries of degradation calibrators for phenotypes with typical (▲) and high (●) degradation.
The mean and SD for recovery are shown for each group of samples.

Although we cannot rule out bacterial contamination before generation, we believe it is highly unlikely, especially considering that month over month, approximately 2% of all plasma renin activity samples exhibit this effect. For a growing number of patients (n > 50) from whom we were able to obtain a second sample, the degradation phenotype was observed in the second sample. In these cases, independent samples were drawn days or weeks apart. In addition, high-degradation samples appear to be randomly distributed, with no dependence on geographic region, clinic, or blood-collection center.

Author Contributions: *All authors confirmed they have contributed to the intellectual content of this paper and have met the following 3 requirements: (a) significant con-*