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Letter to the Editor: A call for transparency in immunoassay techniques to enhance Rigor and Reproducibility

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In the interest of transparency, the first author of this letter (EAS) admitted to co-authors (JGS & JDS) of being the dreaded Reviewer #2 (Tardy, 2019) of their recently-accepted paper. The reviewer struggled deeply because Smith and colleagues (in press) accomplish the goal of being one of the first examinations of hair sex hormones in children; yet, being one of the first raises the bar for the level of detail required to claim novelty of assay techniques. As was hoped, independent reviewers including EAS saw the potential for this paper (Smith, et al., in press) to become a seminal methods piece in an emerging area of hair biomarker assessment. To fulfill that purpose, substantial detail about the assay

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steps and assay protocol validation was needed but, unfortunately, was missing. Using the context of this omission as an illustration, this letter will consider why conflicts regarding the need to enhance reproducibility through rigor and transparency (Collins & Tabak, 2014; Hewitt, Brown, Murphy, Grieder, & Silberberg, 2017) versus protection of intellectual property arise. This letter then concludes with recommendations for navigating industry partnerships within the biomarker field.

Seeking to establish measurement of hair sex hormones in children, Smith and colleagues (in press) initiated discussions with Stratech Scientific, a commercial Salimetrics laboratory based in Australia. Stratech appeared an invested partner, and a verbal agreement culminated in development of hair hormone immunoassays. Recognizing assay development is not a small endeavor, scientific and commercial partners agreed to collaboratively write an empirical report of the pilot data (N=35) and subsequent larger cohort (N=128). During manuscript preparation, Stratech resisted reporting assay details, citing protection of intellectual property. Peer reviewers (including EAS) noted that details needed to replicate biomarker assays were missing from Smith and colleagues (in press), such as the amount of hair minced, method of mincing (e.g., grinder), length and conditions of incubations, quantity of extraction agents and sample buffer. Repeatedly throughout peer-review, the commercial partner made it clear that methodological assay details would not be made available despite Stratech's apparent good intentions at the outset of the collaboration. The manuscript's review process was hampered by these omissions as Smith and colleagues (in press) negotiated with Stratech through the peer-review process, and, ultimately were unable to fulfill reviewer requests for transparency.

This conflict with a commercial entity must be framed according to policies from the National Institutes of Health that have called for enhanced rigor and transparency. These policies have emerged to address concerns that 64-89% of major biomedical findings fail to replicate (Begley & Ellis, 2012; Prinz, Schlange, & Asadullah, 2011). Rigor includes authentication of key biological resources (e.g., antibodies and specialty chemicals) which are sufficiently identified in only about half of publications (Vasilevsky et al., 2013), indicating a broader issue across the biomedical literature. Developmental Psychobiology embraces the call for reproducibility in author guidelines "*Accurate and complete reporting enables readers to fully appraise research, replicate it, and use it.*" Transparency enhances the potential for replication and allows other scholars to evaluate and critique critical steps. Reporting detailed internal validation findings establishes that the assay methodology itself is working and serves as standard practice, pre-requisite steps for rigorous measures and methods (Tholen, Kallner, Kennedy, Krouwer, & Meier, 2004; Tholen et al., 2004).

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What risk exists if Stratech made a mistake in extraction and assay for these novel hair assays? Smith and colleagues(Smith, et al., in press) report the frequency that hair concentrations were below the assay's level of detection. Knowing the assay's upper and lower limit of detection– critical internal validation steps – establishes the concentrations within which the assay can be trusted. Some of the first research in this area, Grotzinger and colleagues (2018a; 2018c) reported concentrations of hair testosterone and DHEA below detection levels in ~1/4th and 1/10th of participants, respectively, using liquid chromatography tandem mass spectrometry (LC-MS/MS). Smith and colleagues (in press) reported comparable DHEA yet lower testosterone concentrations as Grotzinger,(2018a; 2018c) which were within the detectable range of Stratech's ELISA for all participants. This is an interesting partial replication, if assay validation steps hold up, and raises the possibility that immunoassays may be advantageous over LC-MS/MS by having a forgiving lower limit of sensitivity (Gao et al., 2013). This is particularly relevant for child and adolescent sampling, where concentrations are frequently lower. Yet this conclusion is predicated on the assumption that assay values are accurate and validity has been adequately tested – information not currently provided (Smith, et al., in press).

This assumption may not end up harming the field; the commercial lab may have generated reliable hormone concentrations. Stratech is a professional commercial laboratory that processes hundreds of samples per week using a well-regarded immunoassay kit. But consider the recent example of oxytocin to illustrate the importance of rigorous and transparent internal validation. McCullough and colleagues (McCullough, Churchland, & Mendez, 2013) described how older oxytocin studies reported ranges in the low pg/mL range but in the early 2000s a new technique (contra-indicated by kit manufacturers) skipped a time-intensive extraction step. This new protocol was not rigorously validated and the range of oxytocin values now commonly reported were hundreds-fold higher than extracted oxytocin concentrations. More problematic, correlations between these techniques was essentially null and back-validation of non-extracted oxytocin failed to demonstrate basic internal validity. Unfortunately, this calls into question the bulk of findings on peripheral oxytocin for the past 15 years. It may not be headline capturing to report spike-and-recovery results, but it is nonetheless foundational to know if an assay for the 'molecule of trust' can be trusted.

It is naïve to paint transparency and replication as a simple issue given that many technological advances emerge through industry partnerships. Funding agencies encourage such collaborations (e.g., SBIR, STTR funding mechanisms), even budgeting modest profit margins so that for-profit companies are incentivized to take on the challenge of invention. This Reviewer #2 has benefited greatly from such

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partnerships (Miocevic et al., 2017; Shirtcliff et al., 2015) and has learned to navigate non-disclosure agreements, trade secrets, and patent applications. Funding agencies are clear that grant proposals involving industry collaborators should not "give away the goose" but should provide enough detail for reviewers to critically evaluate proposed technology development. *Prior Art* should be used to guide contractual agreements between researchers and commercial laboratories. *Prior Art* defines the scope of an invention's originality, stating that an innovation is not classified as an invention (and thus could be patentable or should be protected by trade secrets) if the innovation has been described previously or where the next steps are obvious to a person of ordinary skill in the field (Cook, Kenny, & Goldstein, 1991). Such is the case with details about extraction protocols and assay steps. Decisions of whether to use 200 versus 400 mL of buffer, to incubate for 18 - 24 hours with or without vibration and/or rotation may be key to establishing the assay's validity, but do not constitute the stroke of ingenuity needed to pass the test of *Prior Art*. Despite considerable time, money, and thankless effort that goes into testing whether the assay works best with 15mg or 25mg of hair, such conclusions should be freely and openly shared with the field (Wang et al., 2019; Wang, Moody, & Shirtcliff, 2016).

Navigating the dissemination and protection of information should continue to be actively debated: the authors are confident that Wiley, the parent company for *Developmental Psychobiology*, has struggled with managing the controversy around open-source information (Choudhury, Fishman, McGowan, & Juengst, 2014; Kratz & Strasser, 2014). It is heartening to see the debate leaning towards open dissemination through official channels regarding open-source science and transparency policies (Begley & Ellis, 2012; Prinz, et al., 2011). Yet perhaps more striking is how opening the unofficial channels of scientific communication can efficiently improve the self-correcting iterative process of science. Consider how Smith and colleagues (in press) responded to a query about whether the unit of measurement for hair sex hormones (pg/mL) fit with the commercial kit's range of sensitivity (10.2-1000 pg/mL for DHEA and 6.1-600 pg/mL for testosterone, Salimetrics, LLC) and if those concentrations were within the expected range of concentrations expected based on prior studies (Grotzinger, et al., 2018a; Grotzinger, et al., 2018c). Rather than shrug off such details, Smith and colleagues corresponded with A. Grotzinger (author JGS, 8/2/2018) and that open dialogue led to an erratum which corrected Grotzinger's lower level of detection from 0.1 pg/mL to 0.1 pg/mg (Grotzinger et al., 2018b). Compared to pg/mg, these hormone concentrations in pg/mL are 6.25 and 25 times higher for DHEA and testosterone, respectively (Wang, et al., 2019, by our estimates based on specific hair extraction protocols). Such efficient open dialogue has helped clarify expected concentrations for hair androgens

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while the literature is still in its nascent stage, not 15 years from now when a typo has become tradition and established the *prior art* in the field.

It is in the spirit of such open dialogue that this letter is written collaboratively between the dreaded Reviewer #2 (Tardy, 2019) and several Smith paper co-authors. Independently, both research groups advocated for this format to stress the value of informal discussions and open scientific discourse. Using this "behind the scenes" description as an example, we hoped to illustrate the importance of applying recent NIH guidelines about collaboration (Connell, 1985), which call for clearly delineating roles and responsibilities, to industry partners and commercial services. Such collaborations are recommended to begin with written agreements that differentiate protected information subsumed within non-disclosure agreements from full methodological disclosure of information[6] not covered by *Prior Art*. It may be in researchers' best interests to educate themselves about unique challenges (and opportunities) of industry partnerships (Tierney, Meslin, & Kroenke, 2016) so as to enhance transparency without disincentivising commercial innovation. It is also the hope of this letter to illustrate the importance of the self-corrective peer review process for enhancing rigor through transparency and ensuring the future potential for replication to those who follow in our wake (Brecht, 1967).

References

Begley, C. G., & Ellis, L. M. (2012). Drug development: Raise standards for preclinical cancer research. *Nature, 483*(7391), 531.

Brecht, B. (1967). An die Nachgeborenen. *Gesammelte Werke, 4*, 722-725.

Choudhury, S., Fishman, J. R., McGowan, M. L., & Juengst, E. T. (2014). Big data, open science and the brain: lessons learned from genomics. [Review]. *Frontiers in Human Neuroscience, 8*(239). doi: 10.3389/fnhum.2014.00239

Collins, F. S., & Tabak, L. A. (2014). NIH plans to enhance reproducibility. *Nature, 505*(7485), 612-613.

Connell, J. P. (1985). A new multi-dimensional measure of children's perception of control. *Child Development, 56*, 1018-1041.

Cook, W. L., Kenny, D. A., & Goldstein, M. J. (1991). Parental affective style risk and family system: A social relations model analysis. *Journal of Abnormal Psychology, 100*, 492-501.

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Gao, W., Stalder, T., Foley, P., Rauh, M., Deng, H., & Kirschbaum, C. (2013). Quantitative analysis of steroid hormones in human hair using a column-switching LC-APCI-MS/MS assay. *J Chromatogr B Analyt Technol Biomed Life Sci, 928*, 1-8. doi: 10.1016/j.jchromb.2013.03.008

Grotzinger, A. D., Briley, D. A., Engelhardt, L. E., Mann, F. D., Patterson, M. W., Tackett, J. L., . . . Harden, K. P. (2018a). Genetic and environmental influences on pubertal hormones in human hair across development. *Psychoneuroendocrinology, 90*, 76-84. doi: 10.1016/j.psyneuen.2018.02.005

Grotzinger, A. D., Briley, D. A., Engelhardt, L. E., Mann, F. D., Patterson, M. W., Tackett, J. L., . . . Paige Harden, K. (2018b). Corrigendum to "Genetic and environmental influences on pubertal hormones in human hair across development" [Psychoneuroendocrinology 90 (2018) 76-84]. *Psychoneuroendocrinology, 98*, 253. doi: 10.1016/j.psyneuen.2018.08.011

Grotzinger, A. D., Mann, F. D., Patterson, M. W., Tackett, J. L., Tucker-Drob, E. M., & Harden, K. P. (2018c). Hair and Salivary Testosterone, Hair Cortisol, and Externalizing Behaviors in Adolescents. *Psychol Sci, 29*(5), 688-699. doi: 10.1177/0956797617742981

Hewitt, J. A., Brown, L. L., Murphy, S. J., Grieder, F., & Silberberg, S. D. (2017). Accelerating Biomedical Discoveries through Rigor and Transparency. *ILAR Journal, 58*(1), 115-128. doi: 10.1093/ilar/ilx011

Kratz, J., & Strasser, C. (2014). Data publication consensus and controversies. *F1000Research, 3*, 94-94. doi: 10.12688/f1000research.3979.3

McCullough, M. E., Churchland, P. S., & Mendez, A. J. (2013). Problems with measuring peripheral oxytocin: can the data on oxytocin and human behavior be trusted? *Neuroscience & Biobehavioral Reviews, 37*(8), 1485-1492.

Miocevic, O., Cole, C. R., Laughlin, M. J., Buck, R. L., Slowey, P. D., & Shirtcliff, E. A. (2017). Quantitative Lateral Flow Assays for Salivary Biomarker Assessment: A Review. *Front Public Health, 5*, 133. doi: 10.3389/fpubh.2017.00133

Prinz, F., Schlange, T., & Asadullah, K. (2011). Believe it or not: how much can we rely on published data on potential drug targets? *Nature reviews Drug discovery, 10*(9), 712.

Shirtcliff, E. A., Buck, R. L., Laughlin, M. J., Hart, T., Cole, C. R., & Slowey, P. D. (2015). Salivary cortisol results obtainable within minutes of sample collection correspond with traditional immunoassays. *Clin Ther, 37*(3), 505-514. doi: 10.1016/j.clinthera.2015.02.014

Smith, J., Johnson, K., Whittle, S., Allen, N., & Simmons, J. (in press). Measurement of cortisol, DHEA, and testosterone in the hair of children: preliminary results and promising indications. *Developmental Psychobiology*.

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Tardy, C. M. (2019). We Are All Reviewer# 2: A Window into the Secret World of Peer Review *Novice Writers and Scholarly Publication* (pp. 271-289): Springer.

Tholen, D. W., Kallner, A., Kennedy, J. W., Krouwer, J. S., & Meier, K. (2004). *NCCLS document EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline* (2nd ed.). Wayne, PA: Clinical and Laboratory Standards Institute.

Tholen, D. W., Linnet, K., Kondratovich, M., Armbruster, D. A., Garrett, P. E., Jones, R. L., . . . Tsai, J. (2004). *NCCLS document EP17-A: Protocols for determination of limits of detection and limits of quantitation: Approved guidelines*. Wayne, PA: Clinical and Laboratory Standards Institute.

Tierney, W. M., Meslin, E. M., & Kroenke, K. (2016). Industry support of medical research: important opportunity or treacherous pitfall? *Journal of general internal medicine, 31*(2), 228-233.

Vasilevsky, N. A., Brush, M. H., Paddock, H., Ponting, L., Tripathy, S. J., Larocca, G. M., & Haendel, M. A. (2013). On the reproducibility of science: unique identification of research resources in the biomedical literature. *PeerJ, 1*, e148-e148. doi: 10.7717/peerj.148

Wang, W., Moody, S. N., Kiesner, J., Appiani, A. T., Robertson, O. C., & Shirtcliff, E. A. (2019). Assay Validation of Hair Androgens Across the Menstrual Cycle. *Psychoneuroendocrinology, 101*, 175-181.

Wang, W., Moody, S. N., & Shirtcliff, E. A. (2016). Noninvasive hair assay for sex hormones: Preliminary protocol validation. *Psychoneuroendocrinology, 71*, 45.

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