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Editorial

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Translational researchers beware! Unreliable commercial immunoassays (ELISAs) can jeopardize your research

With a global market of approximately $1.6 billion per year, antibodies (Abs) and Ab-based assays (e.g., ELISA, immunohistochemistry) represent foremost tools in translational studies [1]. The last decade has witnessed a boost in new biotech companies (mainly based in China, India and Brazil), which offer a wide variety of immunoassays for almost every analyte (even against analytes for which commercial demand should be theoretically small). In such a fast-paced, competitive environment, these new companies, instead of making their own reagents (Abs, antigens), often obtain them from external suppliers. The problem starts when these reagents are sold without having been rigorously validated. In fact, a few suppliers (mainly based in Asia) seem to provide the same reagents to different manufacturing companies, resulting in seemingly different ELISA kits (different vendors and catalogue numbers) which are based on the same Abs. Furthermore, in a globalized setting, where smaller biotech companies are continuously merging with existing ones, it is often extremely difficult to discern the roles of the different parties involved (suppliers, manufacturers and distributors). Under these circumstances, it should not be surprising that the quality standards of many of the newly developed immunoassays could be severely compromised.

The problem of market contamination with poor quality commercial Abs is not new [2, 3]. What is alarming is the extent to which this problem has escalated lately. Berglund et al. validated 5436 commercial antibodies from 51 different antibody providers (during the development of the Human Protein Atlas project) and found that half of the Abs could not pass established quality standards [4]. Expectedly, the problem of poor Ab development resulted in an increasing contamination of the market with unreliable ELISA assays [5, 6]. For instance, Gutiérrez et al. recently reported that an ELISA kit from USCN Life Sciences (Wuhan, China), which was designed to recognize human hemojuvelin, was not able to identify the analyte of interest but rather, an unknown protein, which was subsequently found to be the unrelated antigen, ferritin [7]. In our own recent report on a similar incident, we have shown that a kit purchased from the same company, designed to quantify CUB and Zona Pellucida-Like Domains Protein 1 (CUZD1), was unable to detect the analyte of interest but instead, the kit was quantifying the known ovarian cancer antigen, CA125 [8]. In the past, reports for unreliable ELISAs were mainly related to poor performance (e.g., precision) or possible cross-reactivities with other analyte(s). Based on our extensive experience on ELISA assay development and validation [9], we are aware that no ELISA kit is absolutely immune to cross-reactivity by unknown antigens. What is striking is that the two aforementioned examples, do not constitute cross-reactivity, but rather, recognition of an unrelated (by homology) antigen.

While we do not know how this could have happened during manufacturing, the consequences of such errors can be quite severe. We spent almost 2 years and approximately $500,000 to identify the antigen that the commercial assay for CUZD1 was measuring (CA125) [8]. Incidences like these also highlight additional possible harms, such as rejection of probably promising biomarkers, a situation that calls for a re-examination of such candidates by alternative technologies.

What does all this mean to translational researchers? Investigators around the world should be aware that certain suppliers are releasing with a fast pace ELISA kits of questionable quality. Such products can lead to unfounded conclusions, waste of many months of research and publications that subsequently need to be retracted. Based on the mounting growth of this problem, we encourage a more strict regulation of the antibody-based market even when the product is designated “for research use only”. In addition, there is an urgent need for the creation of independent bodies for standardized antibody validation (efforts towards this goal have already been initiated [10, 11]. Until these needs are met, the best immediate solution for researchers is to avoid purchasing kits from manufacturers that have been reported to produce kits of questionable quality. Table 1 summarizes some measures that should be taken to minimize similar problems in future translational studies.

Table 1 Recommendations to minimize future mishaps with Ab-based translational research.

1. Suggestions for ELISA manufacturers and distributors:
   – Describe immunogen used during Ab development (including sequence of protein/peptide, expression system used, method of antigen purification, carrier proteins, adjuvant, immunization details).
   – Describe antibody production, purification and characterization (even when the Ab was obtained from an external supplier).
   – Specify lot-to-lot consistency in Ab performance.
   – Collaborate closely with researchers if issues arise with your products; be open and transparent.
   – Distributors, always specify who the manufacturer of the kit is.
2. Suggestions for translational researchers:
   – Search for all available Abs against your protein of interest (examples of cross-vendor antibody search tools: Antibody Resource: http://www.antibodyresource.com/, Biocompare: http://www.biocompare.com/).
   – Prefer companies with proven quality record.
   – Perform in-house validation before using commercial antibodies [8].
   – Be critical and do not overlook discrepancies at validation.
   – Report identified problems with commercial Abs or ELISAs.
   – Enter your data in centralized Ab-validation registries (e.g., Antibody Portal: http://antibodies.cancer.gov/apps/site/default; Antibody Validation Database: http://compbio.med.harvard.edu/antibodies/; Antibody Registry: http://antibodyregistry.org/; Antibodypedia: http://www.antibodypedia.com/; CiteAB: http://www.citeab.com/).
3. Suggestions for scientific journals
   – Set guidelines for proper Ab description [12].
   – Do not publish reports that are missing critical info on Ab validation.[a]
   – Encourage researchers to publish reports that deal with unreliable commercial Abs or ELISA assays.

[a]Certain journals have already adopted this policy, including the *Journal of Comparative Neurology,* the *European Journal of Neuroscience, Endocrinology* and all journals of the *Nature Publishing Group*.

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