Switching from RIA to ELISA

Mercodia

Being able to quantify biomarkers in a complex sample such as serum, cell culture media or even urine is of the utmost importance in biomarker research.

Immunoassays that utilize highly specific antibodies to detect biomarkers and analyze their concentration in such samples have been around since 1959 when Yalow and Berson first used radiolabeled insulin to assess the concentration of insulin in humans[1]. This radioimmunoassay (RIA) was followed by a new technique in 1971 when Engvall and Perlman developed an assay where antigens immobilized on a microplate well are incubated with the sample and the concentration of the biomarker of interest quantified using an enzyme-linked anti-immunoglobulin antibody[2]. This method is called enzyme-linked immunosorbent assay (ELISA).

Since then, RIAs and ELISAs have been used extensively to detect and quantify biomarkers. There are, however, differences between the two technologies that should be considered when comparing the two types and selecting an assay format.

His lab made the switch

Assoc. Prof. Dr. Rodrig Marculescu is Head of Endocrinology at the Department of Laboratory Medicine, Medical University of Vienna, Austria, where they perform about one million hormone analyses per year, including about 500 glucagon analyses. His lab has now changed from Glucagon RIA to the Mercodia Glucagon ELISA.
Glucagon is a biomarker of great interest for many scientists in diabetes/obesity/cardiovascular research. However, certain hurdles must be overcome when measuring this molecule. Firstly, glucagon in the blood circulates at very low levels. The method chosen must therefore be sensitive enough to detect these low concentrations. Secondly, the assay format must be reliable. Because a competitive RIA uses an antibody that recognizes the C-terminal of glucagon, the possibility of it measuring proglucagon-derived peptides other than glucagon cannot be ruled out. An independent study has assessed the Mercodia sandwich ELISA and found it to be the most reliable assay for measuring glucagon[3].

Pros and Cons

Both ELISA and RIA can be used to measure glucagon, but the latter format is often based on a polyclonal antibody and may thus show cross-reactivities to substances other than the test analyte, such as hemoglobin and bilirubin. A sandwich ELISA, on the other hand, is based on two antibodies, which ensures high specificity for the analyte. Mercodia ELISA assays are all based on monoclonal antibodies, so reproducibility between lots is assured.

In addition, ELISA's enzyme-based detection system offers the use of antibodies quantities that are higher than those allowed by RIA's radioactive tracer. This makes the ELISA format more robust (lower assay % CVs than RIA) as well as faster (results obtained overnight at the maximum compared to 3-4 days for RIA). The ELISA format also offers excellent performance with small sample volumes.

Rodrig Marculescu describes the process that led up to the decision to change from RIA to ELISA in his lab:

"The glucagon RIA we used before was not suitable for measurements in the lower, non-glucagonoma range, probably due to cross-reactivity to other glucagon-related peptides. Also, we generally strive to replace RIA by more cost-efficient and more automatable assays."

Handling the radioactive chemicals that are necessary in RIA can indeed be problematic. Disposing of radioactive waste is costly,

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and many labs and universities are now totally banning the use of radioactive compounds. In addition, the radioactive isotope used in the RIA format has a much shorter shelf-life than the ELISA assay components.

More specific results

Changing from RIA to ELISA can be a challenge when comparing old and new results, but Rodrig Marculescu believes it is worth it. Put simply, ELISA gives much more specific and accurate results.

“Comparison is hardly possible. RIA has a high unspecific background of about 200 pg/mL. This is an issue in commercial proficiency testing programs, as most laboratories are still using it. In contrast, no method-specific ranges restrict the Mercodia ELISA, which accurately measures glucagon down to physiological basal levels of a few pg/mL.”

In general, Rodrig Marculescu is very pleased with his lab’s change from RIA to ELISA and would definitely recommend the Mercodia Glucagon ELISA to other researchers.

“It is currently, to my knowledge, the only really glucagon-specific immunoassay on the market with an IVD CE label. The biggest advantage with the Mercodia Glucagon ELISA compared to glucagon RIA is its glucagon-specificity, which allows accurate measurement in the physiologic range, plus all the advantages of ELISA compared to RIA in terms of hands-on time andautomatization.”

References

1. Yalow RS and Berson SA, 1959. Nature, 184, 1648-1649.
2. Engvall E and Perlmann P, 1971. Immunochemistry, Sept;8(9):871-4.
3. Wewer Albrechtsen et al, 2014. Diabetologia, 57(9), 1919-1926.