Optimizing the coating procedure of a recAg to Tosyl beads in a CLIA assay

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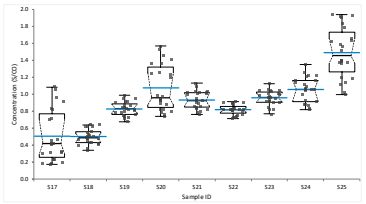
Introduction and Purpose

JSR Life Sciences has a large experience on developing beads and immobilization of antibodies to microparticles.

Biokit is developing a new fully automated chemiluminescent two-step immunoassay for measurement of specific IgG antibodies in human serum or plasma, by coating a specific recombinant antigen (recAg) on paramagnetic microparticles.

During investigation of product development, several paramagnetic microparticles from different brands were tested. For the specific recombinant antigen under study, the most acceptable clinical performance was found when using Tosyl beads from JSR Life Sciences.

However, high variation between reagent lots was observed for some sample levels (around cut-off area):



Sample ID	Global Mean (S/CO)	%CV within lots	Results range (S/CO)
S17	0.51	58.9%	0.17 - 1.08
S18	0.50	17.6%	0.34 - 0.64
S19	0.83	9.7%	0.67 - 0.99
S20	1.08	24.9%	0.74 - 1.57
S21	0.93	10.8%	0.76 - 1.13
S22	0.82	7.2%	0.72 - 0.91
S23	0.96	9.0%	0.77 - 1.13
S24	1.06	13.8%	0.82 - 1.35
S25	1.49	19.8%	1.00 - 1.94

The purpose of the study is to achieve a robust process for the coating of a recombinant antigen to paramagnetic microparticles in an automatic CLIA 2-step assay.

Materials

- Tosyl beads from JSR Life Sciences.
- Recombinant antigen (recAg): around 50 kDa size.

Methods

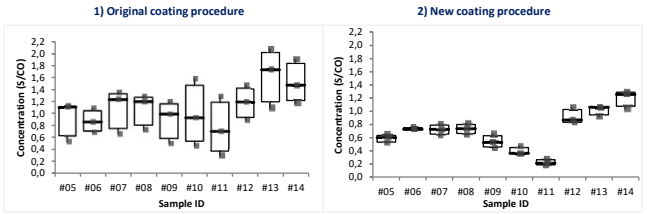
Topic	Investigation
Coating process	Comparison of coating protocols for recAg immobilization: original protocol *vs.* new protocol (addition of a high ionic strength buffer in the coating step and optimization of blocking process).
Assay Performance	Study of impact of new coating protocol on lot-to-lot reproducibility and clinical performance when coating a recAg.

Results

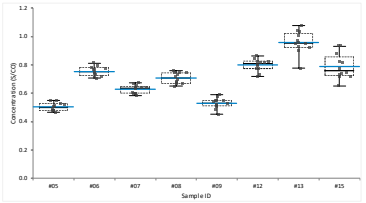
CLIA being a sensitive assay, a variety of parameters and process conditions were investigated to identify impact on performances. A common work between Biokit and JSR Life Sciences allowed an improvement of the situation.

recAg immobilization using high ionic strength buffer in the coating protocol and optimized blocking process

1 lot of recAg was coated to 3 different beads lots by using a 1) original coating procedure and 2) new procedure with addition of high ionic strength buffer and optimized blocking process. Analysis of clinical samples around the cut-off level showed that new coating protocol clearly minimized lot-to-lot variability. Moreover, the clinical status of most of the samples obtained with the new coating method was in line with the reference method.



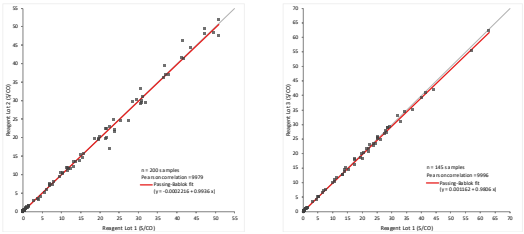
Different lots (3) of recAg were coated to several beads lots (4) in different permutations, using the new coating protocol. The 10 obtained combinations showed high lot-to-lot homogeneity when testing clinical samples around the cut-off level.



Sample ID	Global Mean (S/CO)	%CV within lots	Results range (S/CO)
#05	0.50	5.8%	0.47 - 0.55
#06	0.75	4.6%	0.70 - 0.81
#07	0.63	4.8%	0.58 - 0.67
#08	0.71	5.5%	0.65 - 0.76
#09	0.53	7.1%	0.45 - 0.59
#12	0.80	5.3%	0.72 - 0.86
#13	0.96	8.2%	0.78 - 1.08
#15	0.79	11.4%	0.65 - 0.93

Scale up: 3 reagent lots containing different raw materials were manufactured in large scale by applying the new coating protocol. A large sample panel covering the whole working range was tested and compared between reagent lots. Good reproducibility was observed.

Reagent lot-to-lot comparison with new coating protocol



Clinical performance

Clinical status from reference method of a sample panel were compared with the results obtained with both original and new coating procedures. The assay performance improved in terms of specificity, when applying the new coating protocol.

Conclusions

Immobilization of a recombinant antigen to Tosyl beads from JSR Life Sciences, using a high ionic strength buffer in the coating procedure and applying optimized blocking process, exhibits a substantial improvement on the lot-to-lot reproducibility, showing consistent results for different raw material lots when analyzing real samples. Moreover, clinical performance of the assay is improved in terms of specificity.

The robustness of the coating process makes a reproducible process for a sensitive CLIA 2-step immunoassay.