

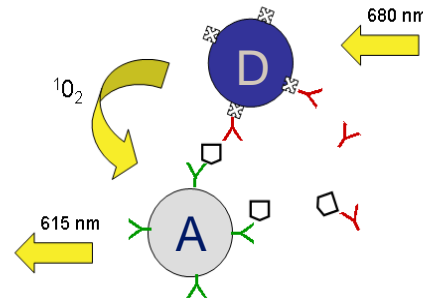
Wash LOCI - An Extension of the LOCI Technology

Fritz Poulsen, MSc, PhD, Scientific Director ADME & Assay Technology, Novo Nordisk

LOCI advantages and limitations

LOCI advantages:

- Good sensitivity.
- Broad working range.
- Small sample volumes.
- Easy to handle.
- Easy to automate.



LOCI limitations:

- PCA require affinity purification based on antigen due to the high percentage of non-immune IgG.
- Potential sample interference.
- Potential hook effect.

LOCI issues

Important to be able to use PCA if only one MCA is available for a sandwich.

PCA are important because a) they are faster to make, b) they give access to many different species, c) they offer more specificities.

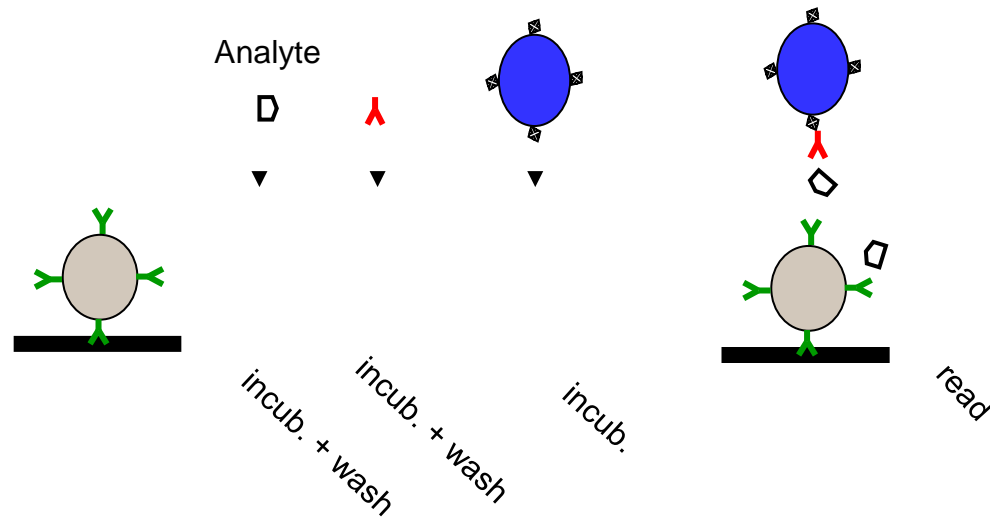
Optimal LOCI assay requires high density of antibody on beads and high concentration of biotinylated antibody.

The antibody density on beads is maximal and can't be increased.

Concentration of biotin antibody can be increased, if biotinylated non-immune IgG can be washed away.

These issues have been addressed just by building a wash step into the LOCI system still keeping the advantages of LOCI.

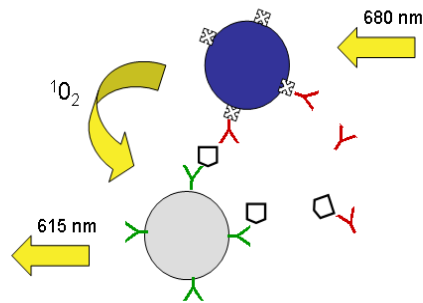
Wash LOCI procedure



- Wash LOCI is a semi-heterogeneous system (only wash after the immunochemical reactions).
- Wash LOCI is run as a 2-step assay to give extra options compared to LOCI.

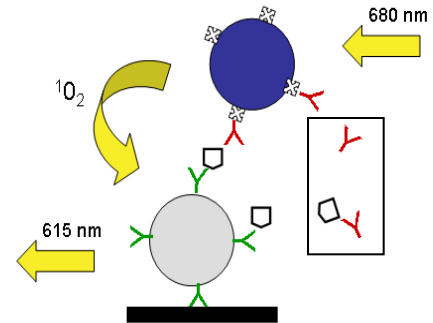
Wash LOCI Principle

LOCI



homogeneous

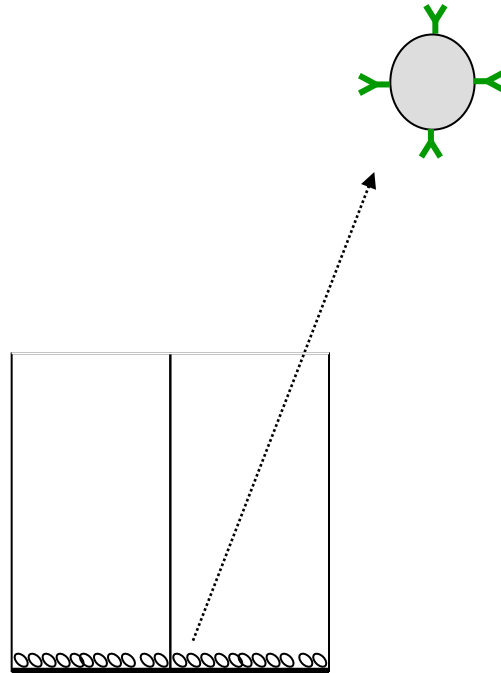
wash LOCI



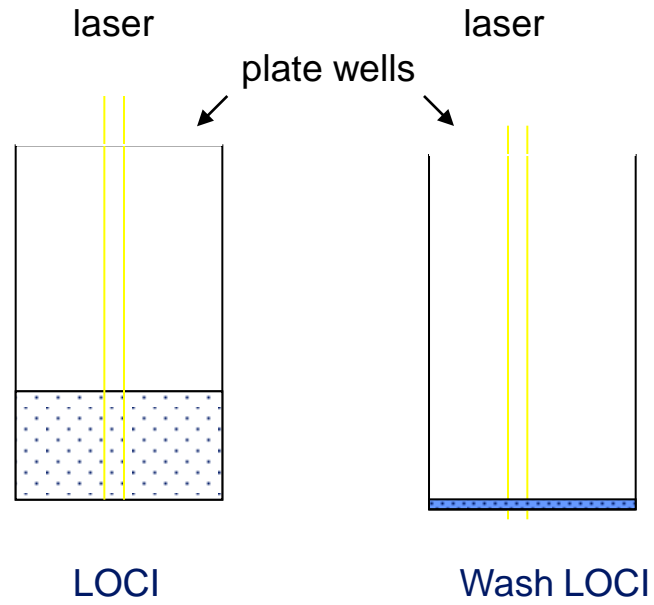
semi-heterogeneous

Solid Phase Coat, Wash LOCI

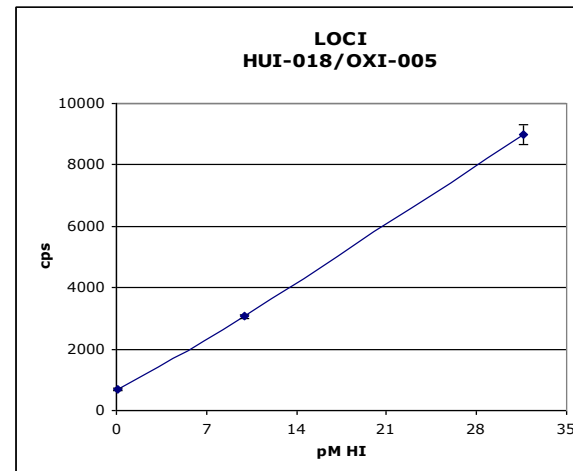
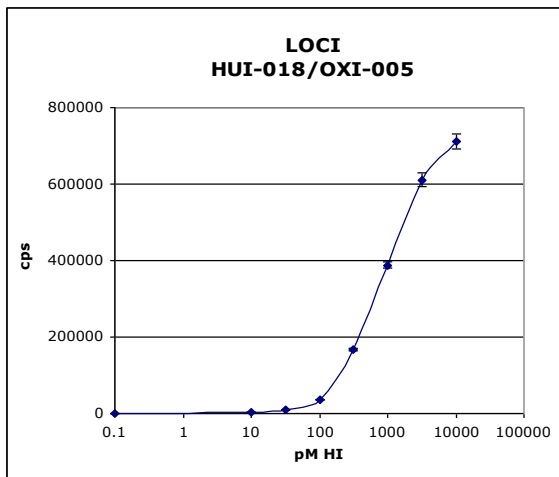
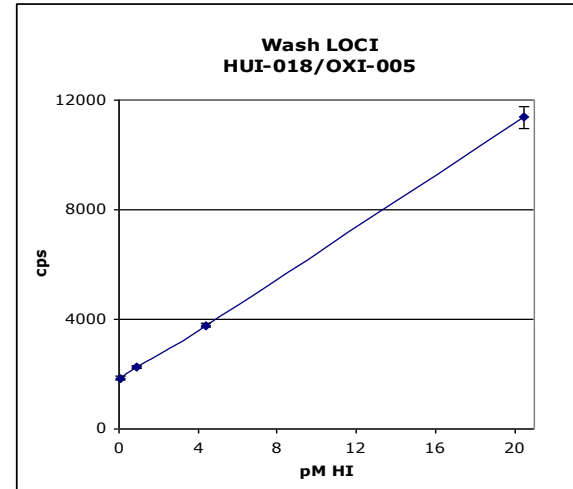
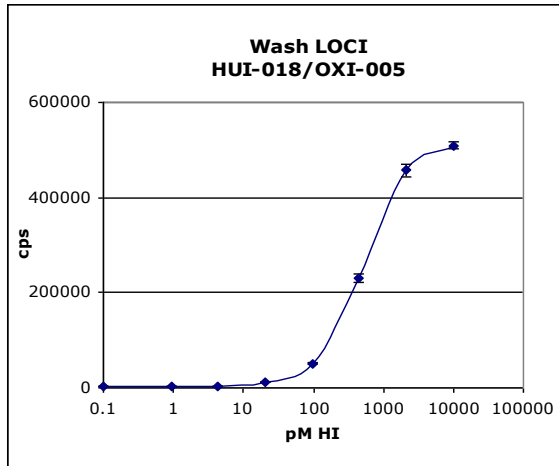
384-well PE-Alpha-plate HB treated



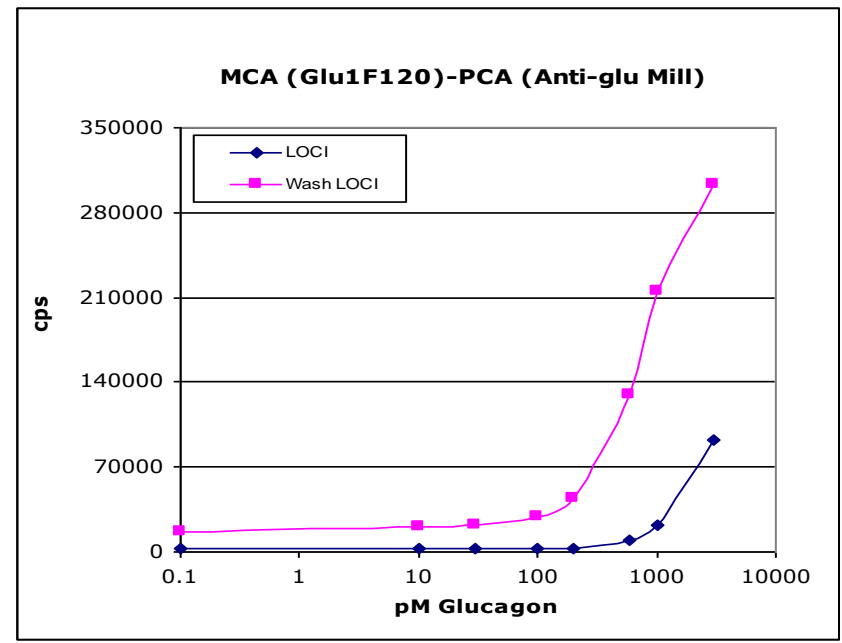
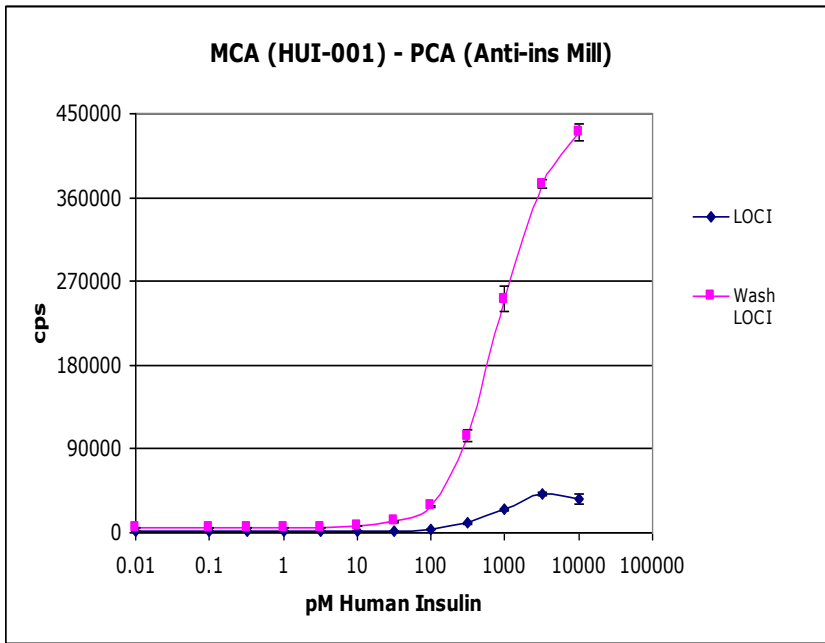
Surface/suspension readout



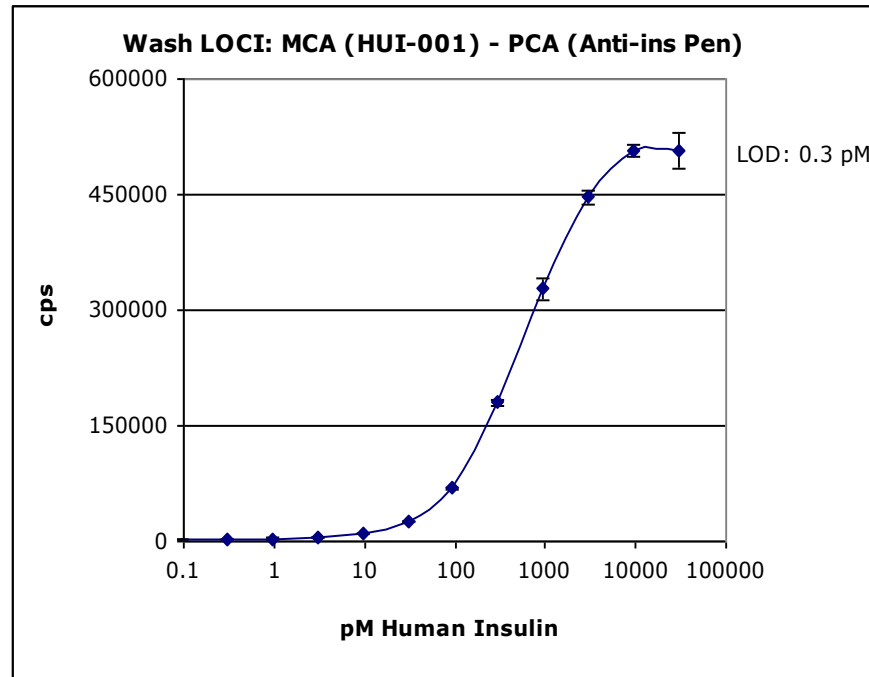
Wash/No wash LOCI MCA/MCA



Wash/No wash LOCI MCA/PCA



Wash/No wash LOCI MCA/PCA



Conclusions wash LOCI

- PCA may be used without antigen-affinity purification.
- PCA with low content of specific antibody may be used.
- Same plates as for LOCI except that plates have been HB treated.
- Same reagents incl. ab-A and SA-D beads as for LOCI.
- Same equipment incl. automation as for LOCI.
- Wash LOCI extends the LOCI technology and makes it even more versatile.

Acknowledgement

Kirsten Borres Jensen
Per Nielsen