Application of a HT magnetic bead based DNA extraction system to diverse mAb process intermediates

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Abstract

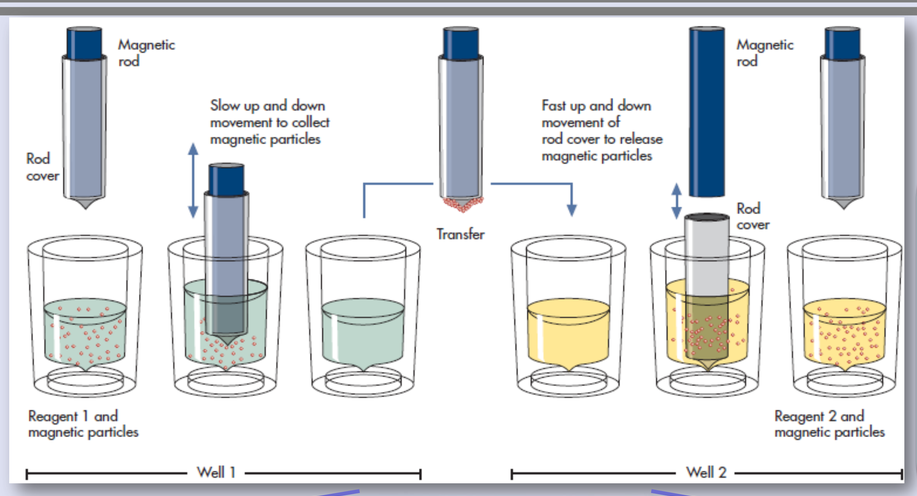
The limit of 100 pg DNA per dose of a therapeutic protein set by regulatory authorities roughly equals the amount of DNA from less than 17 diploid Chinese hamster ovary (CHO) cells. To determine such trace amounts of DNA, the chosen analytical method must be extremely sensitive and robust.

Here, the evaluation and the qualification of a DNA extraction method on magnetic beads are described. The DNA extraction is aided by the use of an automated robotic platform, QIASymphony, prior to quantification by qPCR. This extraction method qualification constitutes an essence to support the biotech process characterization and validation by QbD where the precision and the reproducibility of the analytical method will aid in shaping the production process design space which links CPPs to CQAs.

This study includes tests designed to determine the total precision and accuracy of the extraction, the applicability to various matrices, and the repeatability of the extraction efficiency. Moreover, the range of linearity of the extraction procedure in regards to varying protein and DNA levels was also verified, to account for the range of concentrations of proteins and DNA which can be observed in process characterization studies.

Residual DNA purification using Certal kit

- Prior to processing 500 µL sample are mixed with the same volume of *sample conditioning buffer* to ensure equal performance independent of the sample matrix. No further need to adjust e.g. pH or protein concentration.
- The prepared sample is then processed on the QIAsymphony by sequentially adding proteinase K, lysis buffer, binding buffer and wash buffer
- After processing the sample is eluted in 95 µL and can be directly used for PCR setup
- The pore size of the patented magnetic particles used on the QIAsymphony SP has been optimized to provide a large particle surface area. Macropores allow accessibility for nucleic acid binding, while micropores provide tailor-made bead resuspendability and magnetic response. High binding capacity enables the broad linear range that is necessary to cover the wide range of DNA amounts encountered in typical in-process samples, crude cell harvests, or highly pure final end products.



- *No pre-conditioning required*
- *Proprietary buffer used to condition all the matrices*
- *Only bead transfer, no liquid transfer*
- *Improved Proteinase K*
- *Minimized cross-contamination (UV)*

Inventory check with laser to help eliminate human error

Automated sample transfer from primary tubes

Automated heated lysis and purification

Performances of the QIASymphony

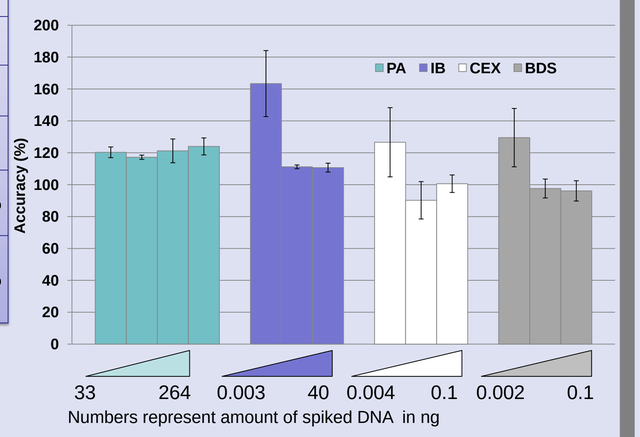
Repeatability and intermediate precision evaluated on all process intermediates of mAb protein: triplicates of extraction in 3 independent Qiasymphony runs

	Process intermediate step				
	Clarified harvest	PA	IB	CEX	BDS
Total Count	9	8	9	9	9
Mean (pg/mL)	40000000	17000	2.0	4.0	2.1
Between (CV%)	5.0%	N/A	13.9%	6.7%	N/A
Repeatability (CV%)	3.3%	27.6%	20.0%	15.1%	18.1%
Intermediate precision (CV%)	6.0%	27.6%	24.4%	16.5%	18.1%

Overall analysis duration:

Sample preparation and extraction:
1 day for 96 samples
Former extraction system: 8 extraction runs
Qiasymphony: **one** extraction run

Accuracy:

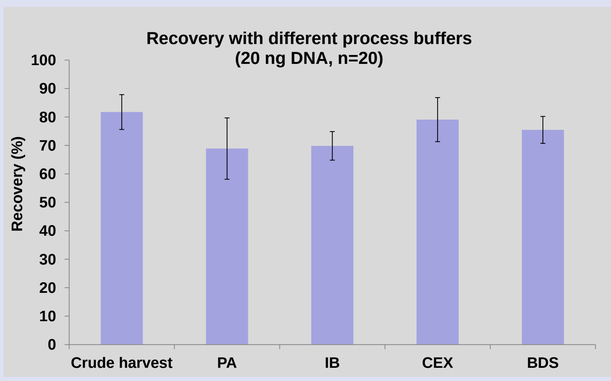


Repeatability
3.3-27.6%
Intermediate precision
6-27.6%

Accuracy is between 80-120% when the DNA content is not too close to the LOQ (< 10 pg/ml)

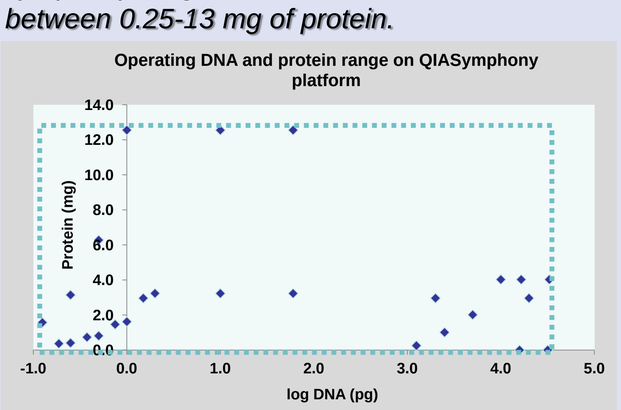
Magnetic bead based DNA extraction application along biotech process

Magnetic bead based extraction system is *applicable* to *all purification process steps* of *mAb process development* in *versatile matrices* of different salt concentration and pH:



Process step	Buffer composition
PA	20mM CH_3COOH, pH 3.2
IB	20mM CH_3COOH, Tris pH 3.2 adjusted to pH 5.75 using Tris
CEX	50mM K_3PO_4, pH 6.7
BDS	10mM sodium citrate, 50mM NaCl, 150mM Sucrose, 0.1% Polysorbate 80 , pH 6.0

QIASymphony magnetic bead based extraction is *linear up to 30 ng of initial DNA load and between 0.25-13 mg of protein.*



- **No exchange of matrix required**
- **Broad linear range; no sample dilution up to 30 ng of initial DNA load**
- **Faster sample prep to accommodate large numbers of process characterization samples**

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