Carryover in the immunoassay laboratory

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Presentation overview

Carryover in the Immunoassay Laboratory

- Regulatory Guidance
  - EMA Guidance for BMV
  - FDA Draft Guidance
- Managing carryover – LC-MS/MS example
- My first experience of carryover
- Platforms affected by carryover
  - Gyrolab
  - Singulex Erenna
- Other assay systems to consider
- Discussion

Guidance from the regulators: LC-MS/MS

- EMA Guidance: Carry-over
  - "Carry-over should be addressed and minimised during method development. During validation carry-over should be assessed by injecting blank samples after a high concentration sample or calibration standard at the upper limit of quantification. Carry over in the blank sample following the high concentration standard should not be greater than 20% of the lower limit of quantification (LLOQ; see below) and 5% for the internal standard. If it appears that carry-over is unavoidable, study samples should not be randomised. Specific measures should be considered, tested during the validation and applied during the analysis of the study samples, so that it does not affect accuracy and precision. This could include the injection of blank samples after samples with an expected high concentration, before the analysis of the next study sample."

Guidance from the regulators: ligand binding assays

- EMA Guidance

  7.1.1.4. Carry-over effect

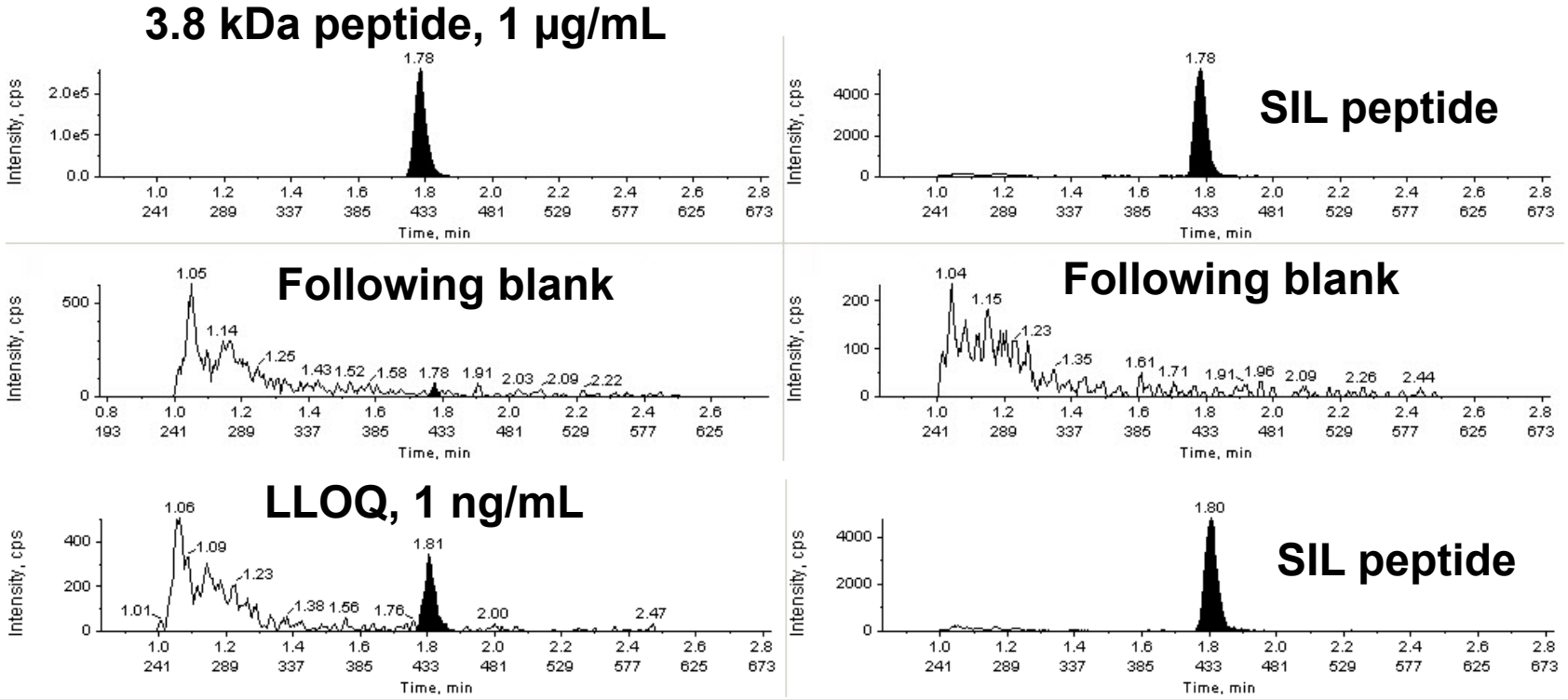
  “If robotic liquid handling systems are used, potential for carry-over should be investigated by placing blank samples after samples with a high analyte concentration or calibration standard at the upper limit of quantification.”

- FDA Draft Guidance (LBA Section)

  “Carryover should be assessed and monitored during analysis. If carryover occurs, it should be mitigated or reduced.”

Carryover in LC-MS/MS

- Carryover assessed by running blanks after high concentration samples
- Assessed as % area compared to that seen for the LLOQ:
  - <20% of LLOQ is target on first blank after STD 8
  - Otherwise managed by multiple blanks

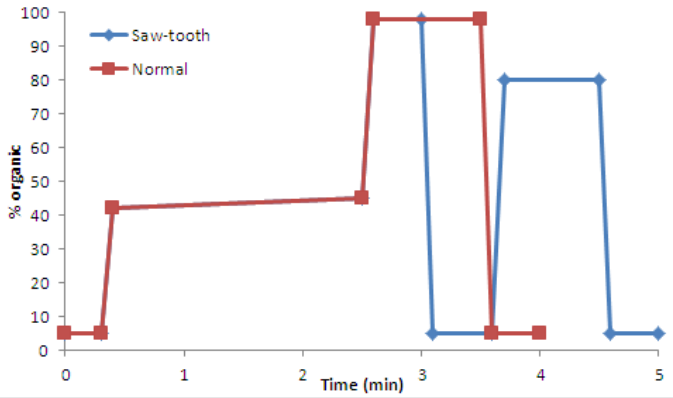


Strategies for removing carryover

- Injection mode
  - Partial loop is usually better than partial loop with needle overfill

- Carryover is not necessarily due to injection system
- Can be adsorption in the system
  - e.g. column carryover

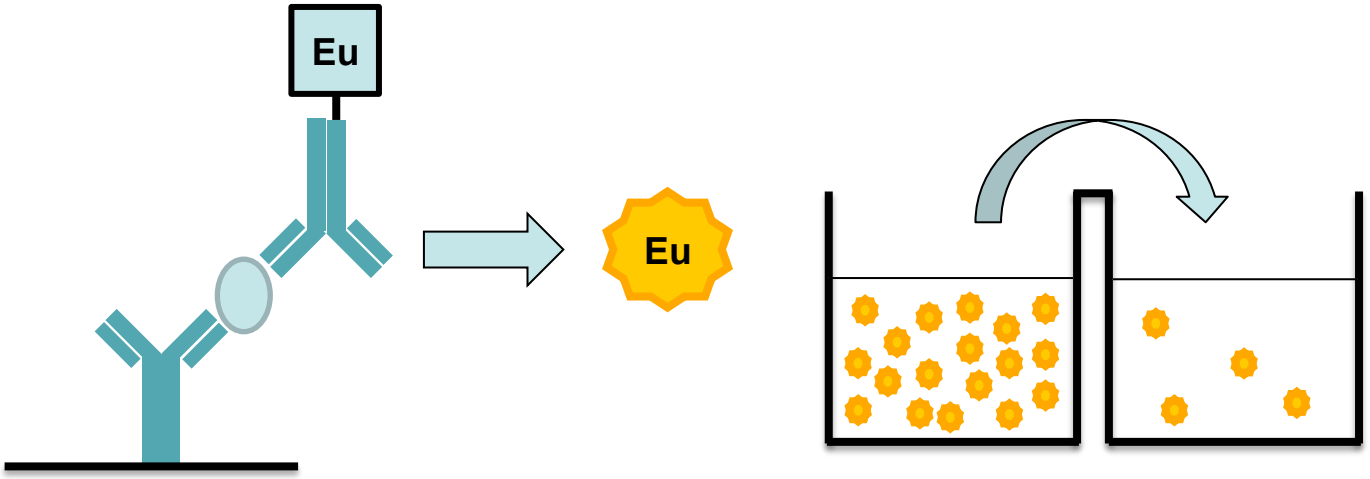
- Saw tooth gradient can help clean system



	% area of LLOQ	
	Saw-tooth	Normal
STD 1	100	100
Blank 1	8.4	65.3
Blank 2	0.0	19.3
Blank 3	0.0	11.6

My first carryover

- Impact of Assay Procedure
  - DELFIA assay



- How does this impact the assay?
  - Poor % CV between replicates
    - Noted mainly for LQC or blank

My first carryover

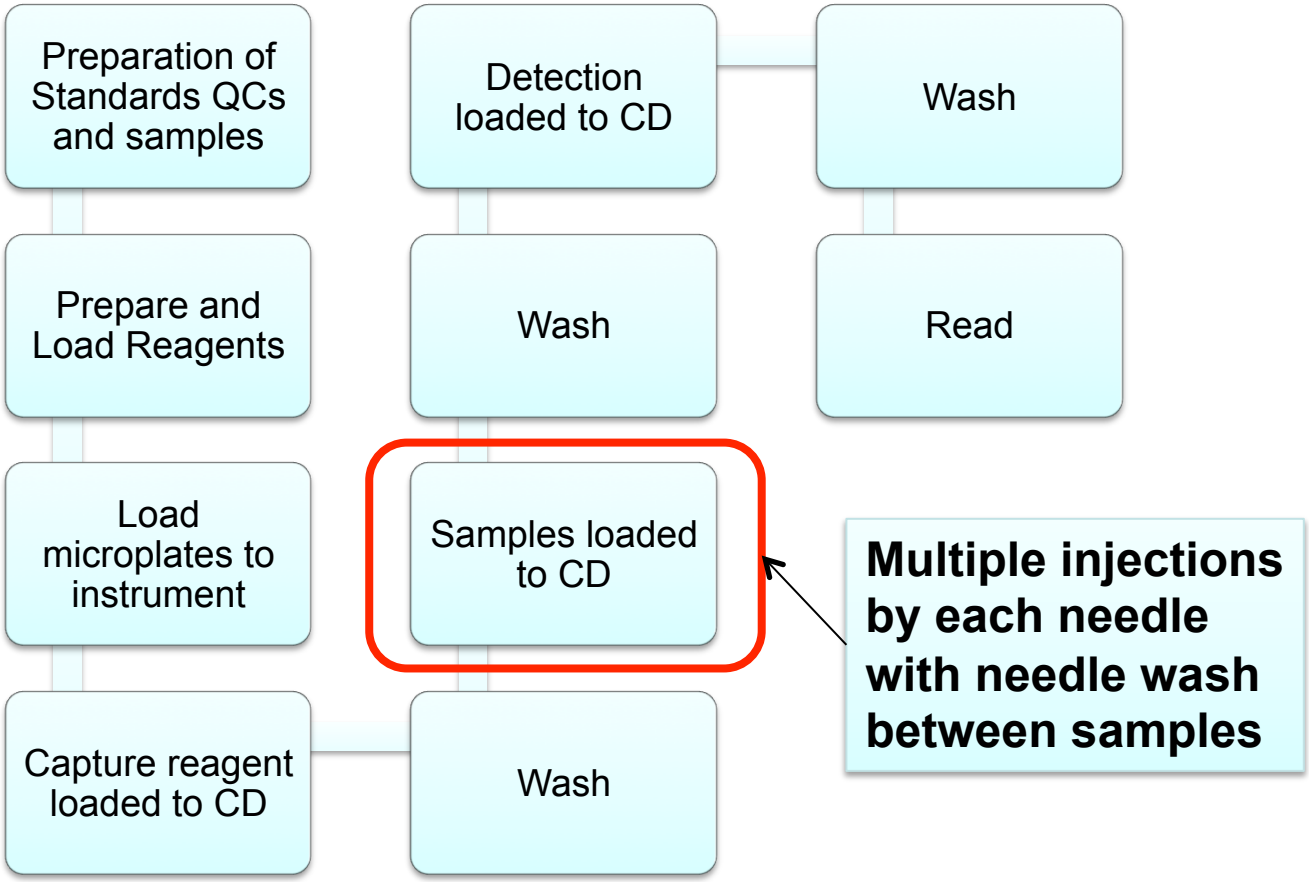
- Problem resolved by:
  - Fresh tips for dispense to each column
  - Not touching tips to the side of the well

- Similar carryover can be observed for standard ELISA or homogenous assays.
  - Good pipetting practice

- Strip well automated washer carryover?
  - Typically indicates a more robust wash procedure is required
  - Consider modification to the wash protocol
    - E.g. modify aspirate/dispense rate
  - Use a 96-well head washer

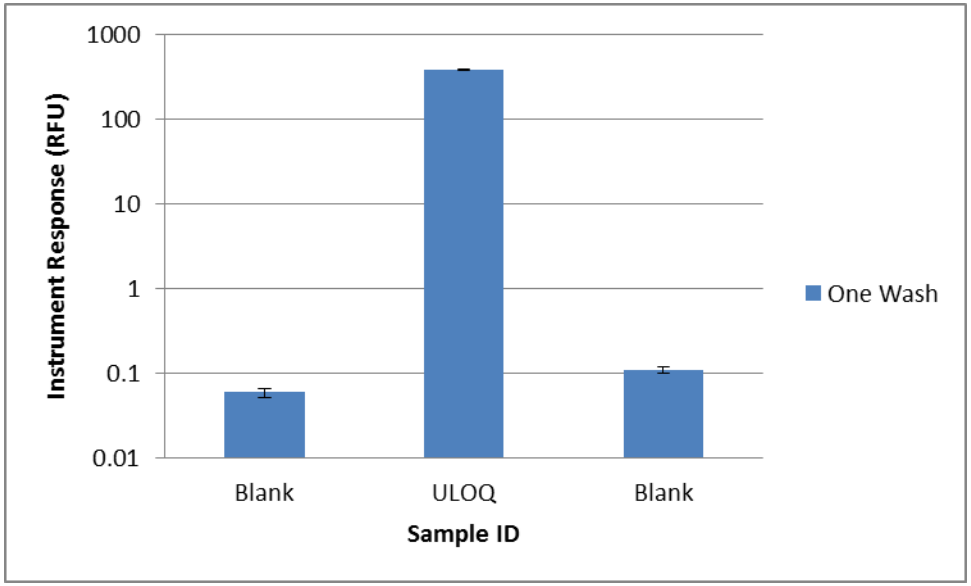
Platforms affected by carryover: Gyrolab workstation

- Identification of the key risk point in Gyrolab workflow



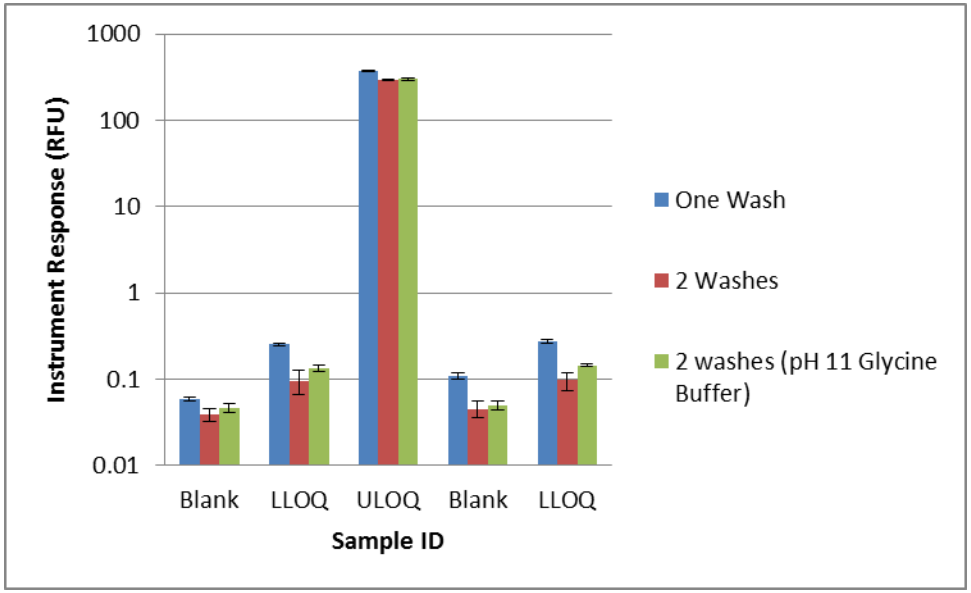
Platforms affected by carryover: Gyrolab Workstation

- 5 Needle System
  - Assess each needle
- Carryover test
  - Injection system carryover
  - Blank – High – Blank test
  - Carryover identified: 1.8-fold change in blank



Platforms affected by carryover: Gyrolab Workstation

- Troubleshooting: Introduce additional needle wash cycle

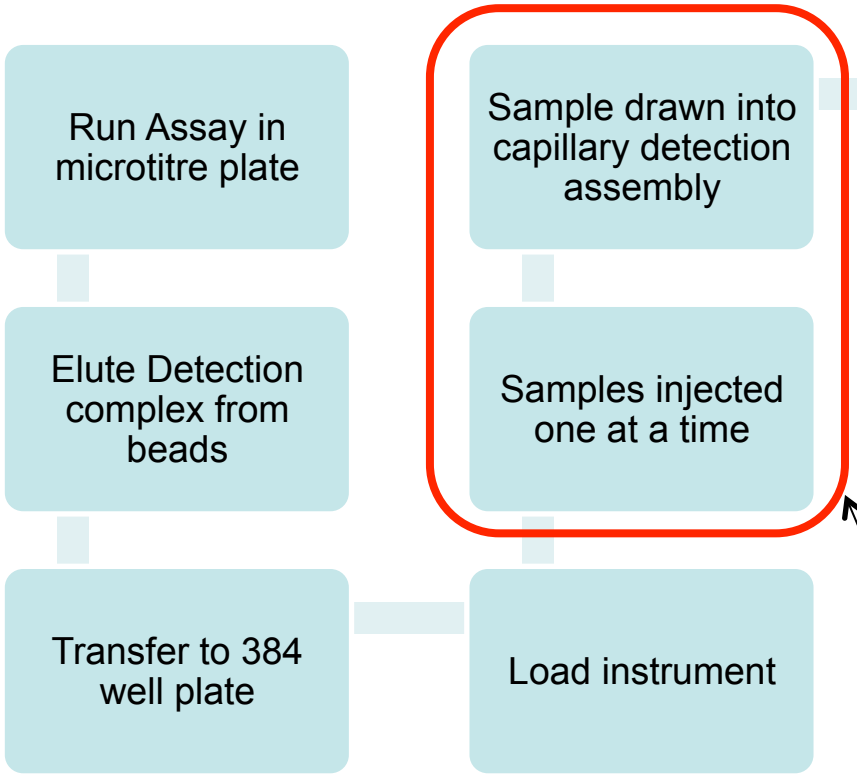


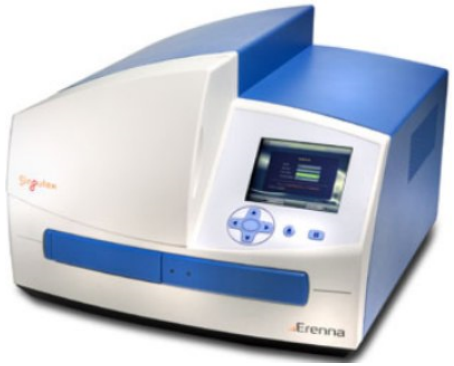
- Added LLOQ sample to monitor for change in sensitivity.
- Further changes possible:

- Salt - Detergent - pH - Assay Diluent

Platforms affected by carryover: Singulex Erenna

- Single needle, injection port and capillary

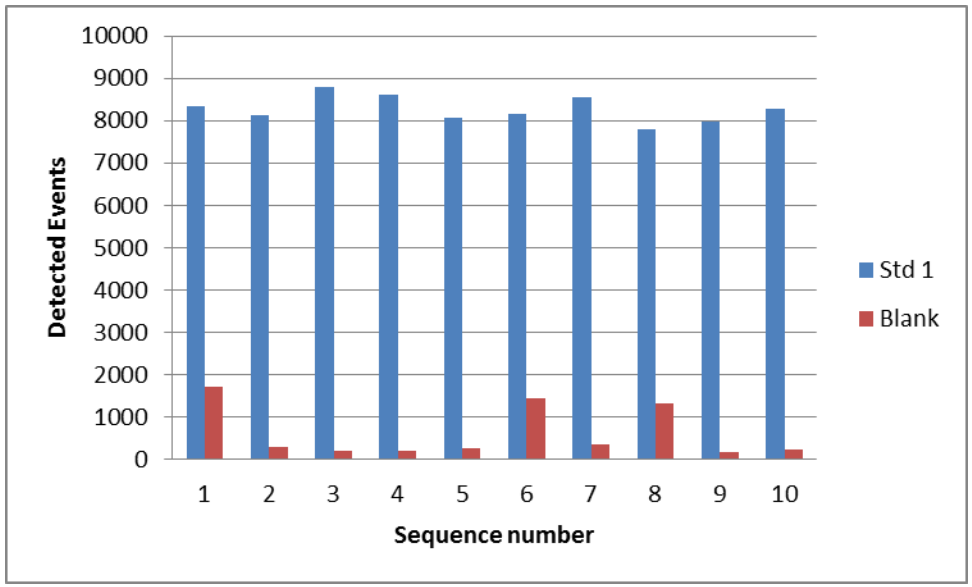


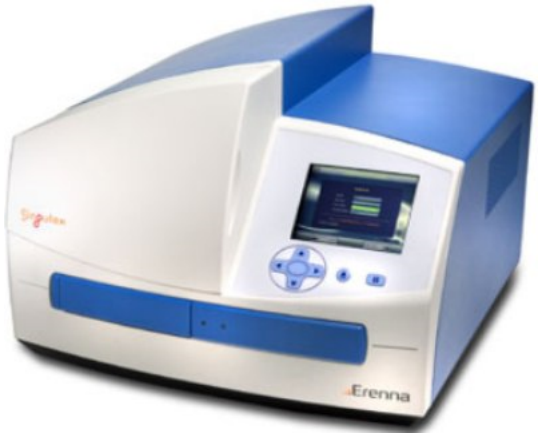


- **Multiple injections by each needle with needle wash between samples.**
- **Capillary flushed with system buffer between samples**

Platforms affected by carryover: Singulex Erenna

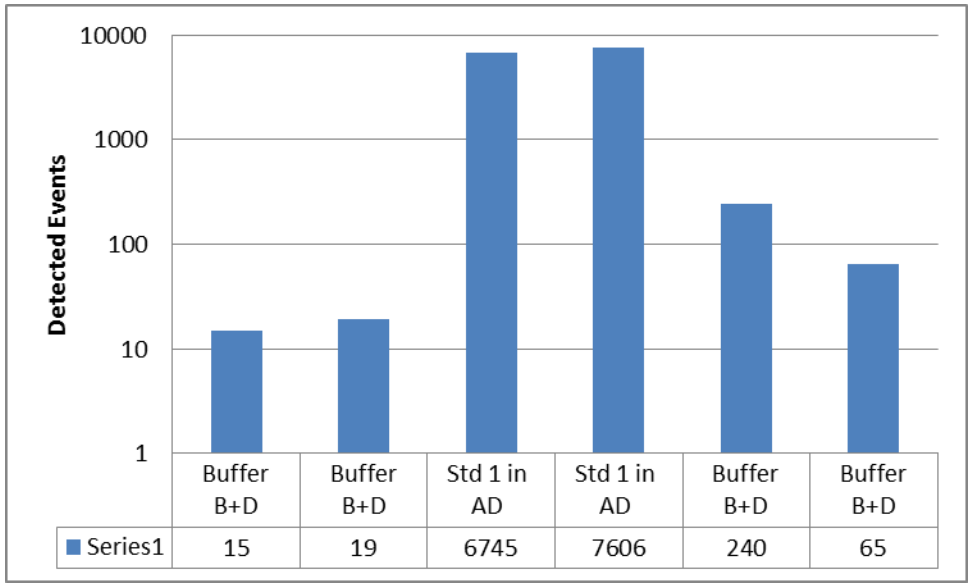
- Carryover identified in method development
  - Carryover test using Blank-High-Blank approach
  - Variable carryover within a run

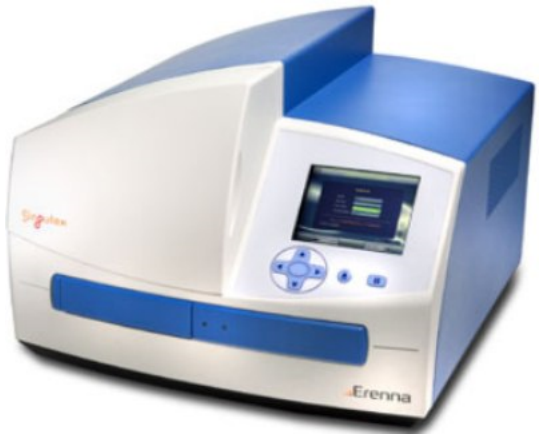




Platforms affected by carryover: Singulex Erenna

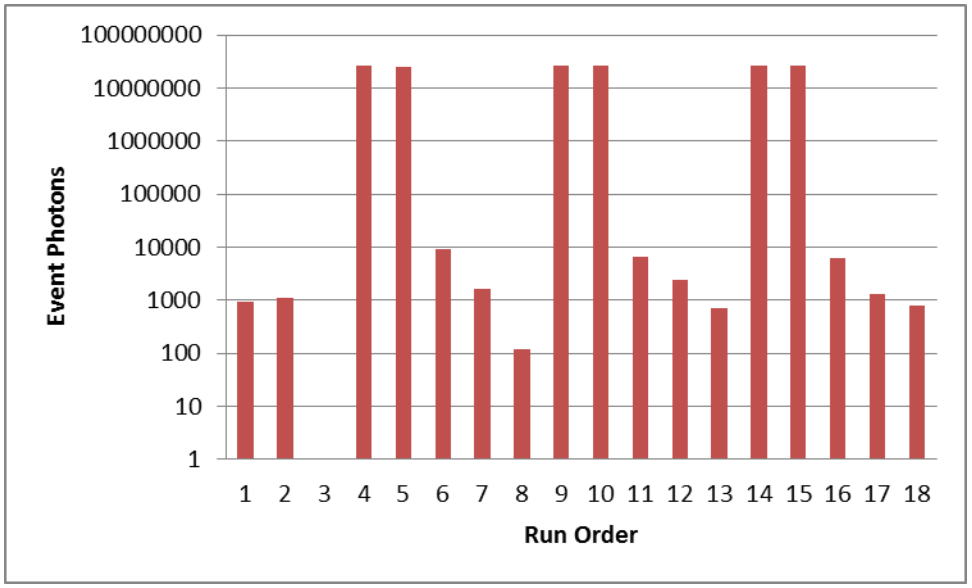
- Further Investigation
  - Impact of duplicate injections
  - Carryover reduced after first blank injected
  - Carryover to second replicate also observed

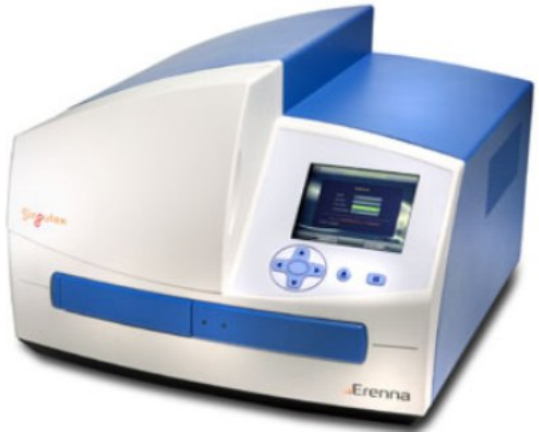




Platforms affected by carryover: Singulex Erenna

- Instrument carryover testing using stock IgG-fluorophore solution
  - Trend Analysis showed an increase in carryover over a 2-3 month period

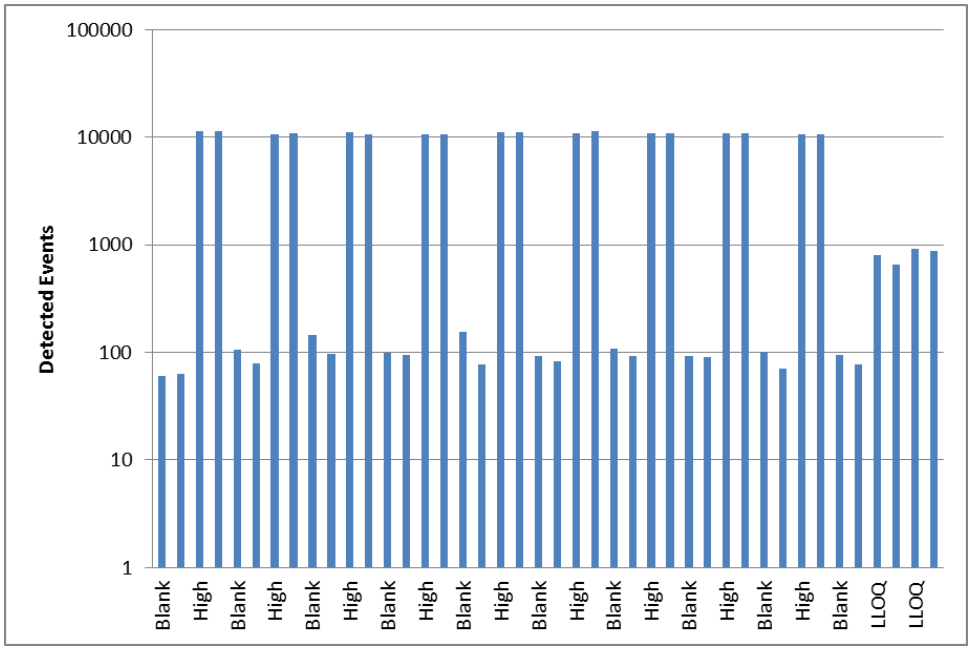


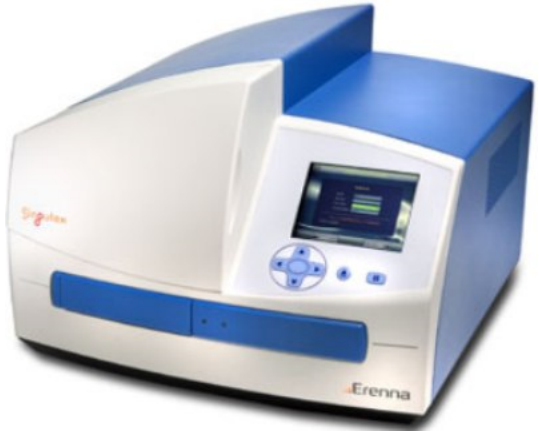


- Troubleshooting included refining peak tubing junctions, replacing the instrument capillary, replacing the injection port and improving needle alignment within the port.

Platforms affected by carryover: Singulex Erenna

- Repeat Carryover Test

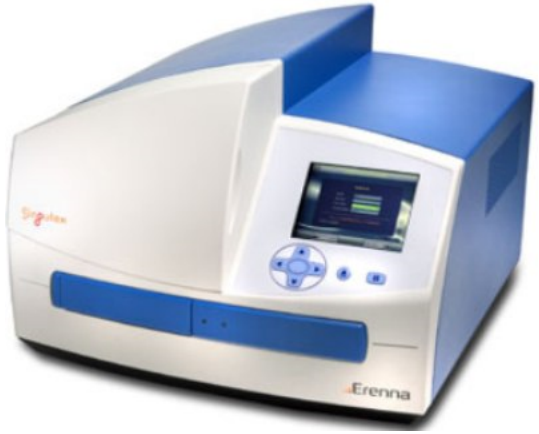




- Extent of carryover not sufficient to affect LLOQ
- Assay developed is 65-fold more sensitive than previous format

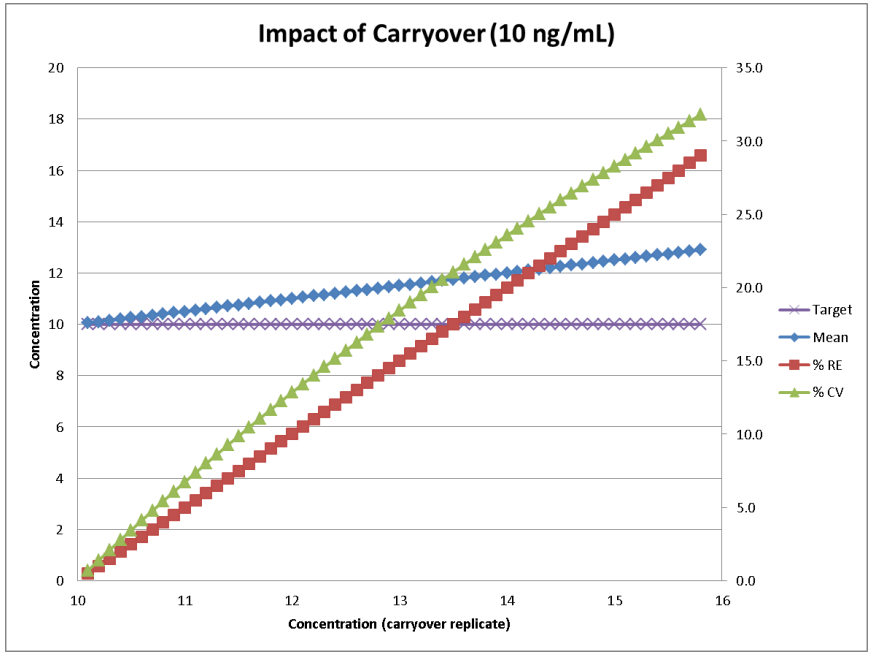
Platforms affected by carryover: Singulex Erenna

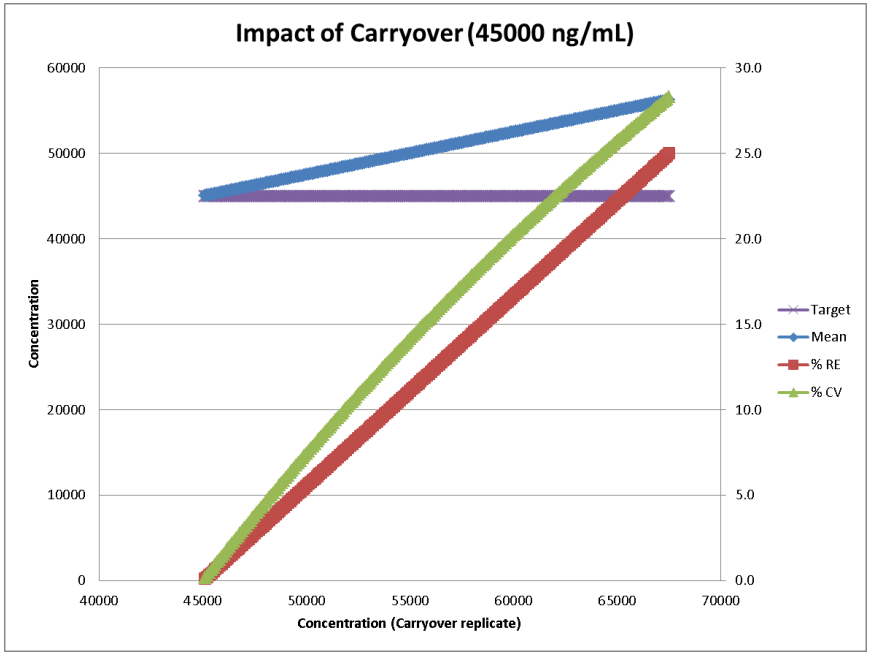
- Further Options for resolving carryover
  - Accumulation of fluorophore and other assay components in capillary?
  1. Modify System Buffer
  2. Dilute out eluted samples – inject less sample
  3. Rejection criteria based on previously injected sample
  4. Run Calibrators low-high then blank
  5. Implement blank injection between each sample
     - Describe the process in your protocol



Impact of carryover on acceptance criteria

- Theoretical example increasing concentration of first replicate
  - % CV will fail at the 20% acceptance criterion before % RE
  - Sample affected by carryover is rejected before concentration is altered by 20%
  - What is the impact of bias on PK/TK, PD or Immunogenicity?





Other platforms to consider

- Any system utilizing a single injection source or single detection channel has a potential risk for carryover

- Automation
  - Large pipetting platforms and automated ELISA systems
    - Avoid use of fixed tips
    - Off-line handling of high concentration stocks

- Clinical Analysers
- SPR
- Flow Cytometers / Luminex
- Bioscale Vibe

Discussion

- Carryover can be a significant issue in the immunoassay laboratory
- Standard microplate assays probably not very high risk
- Risk Assessment of each assay
  - Dynamic Range of assay platform
  - What is the sample path in your platform
  - What are the points of control
    - Can you alter wash/prime reagents
      - Pick reagents based on protein chemistry
    - Can you inject reduce the in-well/in-instrument concentration
    - Add additional control through run design
- A single assessment may not be enough
- Ultimately, does the level of carryover affect accuracy/precision at your LLOQ or expected range?
  - Can you demonstrate acceptance criteria that control for carryover

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Thank you