# REVOLUTIONARY SCREENING OF RESIDUES IN RAW MILK USING THE INFINIPLEX FOR MILK BIOCHIP ARRAY KIT

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## INTRODUCTION

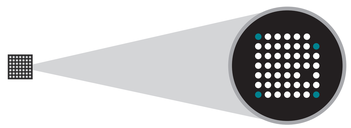
Comprehensive detection of veterinary drugs in milk, including all legislated antibiotics at or below relevant regulatory requirements, is important for consumer protection. Based on biochip array technology, the Infiniplex for Milk (IPM) biochip array allows simultaneous screening of 129 drug residues including mycotoxins, growth promoters, corticosteroids, anti-inflammatories, anti-parasitic and antibiotics per milk sample.
The purpose of this study was to demonstrate the robustness of the IPM array kit in the multiplex screening of Raw Farm Milk (RFM) and Raw Tanker Milk (RTM) followed by LC-MS/MS confirmation (at ILVO).

## METHODOLOGY

### BIOCHIP ARRAY SCREENING

The biochip array kit Infiniplex for Milk (EV4076, Randox Food Diagnostics, Crumlin, UK) was used. Simultaneous competitive chemiluminescent immunoassays defining discrete test sites on the biochip surface were employed and applied to the Evidence Investigator analyser (EV3602, Randox Food Diagnostics, Crumlin UK) which incorporates dedicated software for results processing. Up to 48 samples can be analysed within 2 hours. 23 RFM and 153 RTM samples were screened followed by LC-MS/MS confirmation. Neat milk samples (25µL) were added directly to the biochips.

### Infiniplex for Milk High Density Biochip Array



Example : InfiniPlex for Milk (EV4076)

43 discrete test regions on each biochip

Specific Assay

Generic Assay (ie. detects 2 or more residues)

### Biochip Array Layout

| | | | | | | |
|---|---|---|---|---|---|---|
| REFERENCE QC | TOBRAMYCIN | BAQUILOPRIM | 5-HYDROXY FLUNIXIN | VIRGINIAMYCIN | RIFAXIMIN | ORIENTATION QC |
| PIRLIMYCIN | CHLORMADINONE | SPECTINOMYCIN | QUINOLONES | APRAMYCIN | PHENYLBUTAZONE | CORRECTION QC |
| RACTOPAMINE | METAMIZOLE | SULPHONAMIDES | STREPTOMYCIN | SULPHAGUANIDINE | ERYTHROMYCIN | SULPHAPYRIDINE |
| TETRACYCLINES | LINCOMYCIN | TYLOSIN | CORTICOSTEROIDS | NEOMYCIN | PREDNISOLONE | BETA-LACTAMS |
| TRIMETHOPRIM | AMPHENICOLS | SULPHAMETHAZINE | MELOXICAM | TOLFENAMIC ACID | | HYGROMYCIN B |
| SPIRAMYCIN | NOVOBIOCIN | CEFUROXIME | BACITRACIN | DAPSONE | CEPHALEXIN | MELAMINE |
| CORRECTION QC | POLYMIXINS | KANAMYCIN | NITROXYNIL | AFLATOXIN M1 | GENTAMICIN | CORRECTION QC |

This biochip array has the capability to test for 129 drug residues.

### CONFIRMATORY METHODOLOGY

The LC system consisted of an Aquity UPLC. Separation was achieved on a Kinetex C18 2.1 x 100 mm, 1.7 µm column, injection volume 5µL, flow rate, 0.4 mL/min of $H_2O$/ACN (95/5%) + 0.3% AA. The MS equipment consisted of a Xevo TQ-MS (Waters). Analytes were determined with tandem electrospray positive or negative MS with two transitions. An Obelisc R (Sielc) 2.1 x 100 mm, 5 µm column was used for aminoglycosides separation. The column was held at 40°C, the injection volume was 10 µL and the eluent flow was at 0.5 mL/min. The elution was performed gradually with changing amounts of $H_2O$ + 1 % FA and ACN + 1 % FA.

## RESULTS

### SAMPLE ASSESSMENT

9 (34.6%) RFM and 2 (1.3%) RTM samples screened positive on IPM biochip array for at least one drug residue, including gentamicin, kanamycin, spectinomycin, cefquinome, cephalexin and florfenicol, which were confirmed by LC-MS/MS (florfenicol required LC-MS/MS confirmation by an ISO17025 accredited laboratory).

**RFM Samples**

| | F4 | | F5 | |
|---|---|---|---|---|
| | IPM Array Kit | LC-MS/MS | IPM Array Kit | LC-MS/MS |
| Amphenicols | POSITIVE (>0.4 ppb) | Florfenicol 3.4 ppb* | POSITIVE (>0.4 ppb) | Florfenicol 2 ppb* |
| Kanamycin | POSITIVE (>4 ppb) | 15 ppb | POSITIVE (>4 ppb) | 54 ppb |

| | F6 | |
|---|---|---|
| | IPM Array Kit | LC-MS/MS |
| Beta-lactams | POSITIVE (>6.3 ppb) | Cefquinome 1454 ppb |

| | C2 | |
|---|---|---|
| | IPM Array Kit | LC-MS/MS |
| Gentamicin | POSITIVE (>8.3 ppb) | Gentamicin traces |

| | C6 | | C9 | |
|---|---|---|---|---|
| | IPM Array Kit | LC-MS/MS | IPM Array Kit | LC-MS/MS |
| Beta-lactams | POSITIVE (>0.35 ppb) | N/A** | POSITIVE (>0.35 ppb) | N/A** |

| | P5 | |
|---|---|---|
| | IPM Array Kit | LC-MS/MS |
| Beta-lactams | POSITIVE (>0.35 ppb) | Could not be performed in this matrix |
| Cephalexin | POSITIVE (>23 ppb) | 26.7 ppb |
| Kanamycin | POSITIVE (>4 ppb) | 575 ppb |
| Tobramycin | POSITIVE (>7 ppb) | N/A*** |

| | P6 | |
|---|---|---|
| | IPM Array Kit | LC-MS/MS |
| Kanamycin | POSITIVE (>4 ppb) | N/A** |

| | P7 | |
|---|---|---|
| | IPM Array Kit | LC-MS/MS |
| Spectinomycin | POSITIVE (>3 ppb) | 92 ppb |

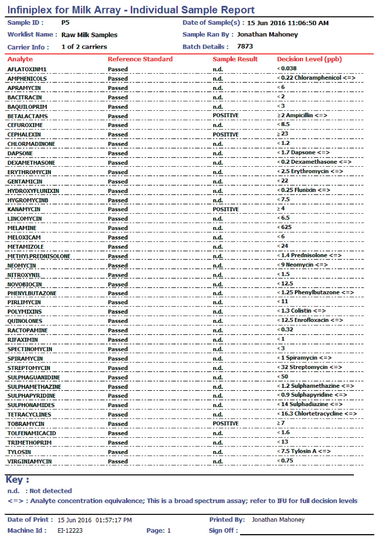
15,500,641; 16,506,641
* LC-MS/MS confirmation by external laboratory accredited ISO 17025
** N/A: insufficient sample available
*** N/A: positive response triggered due to high concentration of kanamycin in the sample.

**RTM Samples**

| | T13 | |
|---|---|---|
| | IPM Array Kit | LC-MS/MS |
| Kanamycin | POSITIVE (>4 ppb) | Kanamycin traces |

| | T63 | |
|---|---|---|
| | IPM Array Kit | LC-MS/MS |
| Beta-lactams | POSITIVE (>0.35 ppb) | N/A** |

### Example of the individual sample report with the IPM biochip platform for RFM sample 5



**Infiniplex for Milk Array - Individual Sample Report**

Sample ID : P5 — Date of Sample(s) : 15 Jun 2016 11:06:50 AM
Worklist Name : Raw Milk Samples — Sample Ran By : Jonathan Mahoney
Carrier Info : 1 of 2 carriers — Batch Details : 7873

| Analyte | Reference Standard | Sample Result | Decision Level (ppb) |
|---|---|---|---|
| AFLATOXIN M1 | Passed | n.d. | < 0.038 |
| AMPHENICOLS | Passed | n.d. | < 0.22 Chloramphenicol <=> |
| APRAMYCIN | Passed | n.d. | < 6 |
| BACITRACIN | Passed | n.d. | < 2 |
| BAQUILOPRIM | Passed | n.d. | < 3 |
| BETALACTAMS | Passed | POSITIVE | > 2 Ampicillin <=> |
| CEFUROXIME | Passed | n.d. | < 8.5 |
| CEPHALEXIN | Passed | POSITIVE | > 23 |
| CHLORMADINONE | Passed | n.d. | < 1.2 |
| DAPSONE | Passed | n.d. | < 1.7 Dapsone <=> |
| DEXAMETHASONE | Passed | n.d. | < 0.2 Dexamethasone <=> |
| ERYTHROMYCIN | Passed | n.d. | < 2.5 Erythromycin <=> |
| GENTAMICIN | Passed | n.d. | < 22 |
| HYDROXYFLUNIXIN | Passed | n.d. | < 0.25 Flunixin <=> |
| HYGROMYCINB | Passed | n.d. | < 7.5 |
| KANAMYCIN | Passed | POSITIVE | > 4 |
| LINCOMYCIN | Passed | n.d. | < 6.5 |
| MELAMINE | Passed | n.d. | < 625 |
| MELOXICAM | Passed | n.d. | < 6 |
| METAMIZOLE | Passed | n.d. | < 24 |
| METHYLPREDNISOLONE | Passed | n.d. | < 1.4 Prednisolone <=> |
| NEOMYCIN | Passed | n.d. | < 9 Neomycin <=> |
| NITROXYNIL | Passed | n.d. | < 1.5 |
| NOVOBIOCIN | Passed | n.d. | < 12.5 |
| PHENYLBUTAZONE | Passed | n.d. | < 1.25 Phenylbutazone <=> |
| PIRLIMYCIN | Passed | n.d. | < 11 |
| POLYMIXINS | Passed | n.d. | < 1.3 Colistin <=> |
| QUINOLONES | Passed | n.d. | < 12.5 Enrofloxacin <=> |
| RACTOPAMINE | Passed | n.d. | < 0.32 |
| RIFAXIMIN | Passed | n.d. | < 1 |
| SPECTINOMYCIN | Passed | n.d. | < 3 |
| SPIRAMYCIN | Passed | n.d. | < 1.5 Spiramycin <=> |
| STREPTOMYCIN | Passed | n.d. | < 12 Streptomycin <=> |
| SULPHAGUANIDINE | Passed | n.d. | < 50 |
| SULPHAMETHAZINE | Passed | n.d. | < 1.2 Sulphamethazine <=> |
| SULPHAPYRIDINE | Passed | n.d. | < 0.9 Sulphapyridine <=> |
| SULPHONAMIDES | Passed | n.d. | < 14 Sulphadiazine <=> |
| TETRACYCLINES | Passed | n.d. | < 16.3 Chlortetracycline <=> |
| TOBRAMYCIN | Passed | POSITIVE | > 7 |
| TOLFENAMICACID | Passed | n.d. | < 1.6 |
| TRIMETHOPRIM | Passed | n.d. | < 13 |
| TYLOSIN | Passed | n.d. | < 7.5 Tylosin A <=> |
| VIRGINIAMYCIN | Passed | n.d. | < 0.75 |

Key :
n.d. : Not detected
<=> : Analyte concentration equivalence; This is a broad spectrum assay; refer to IFU for full decision levels

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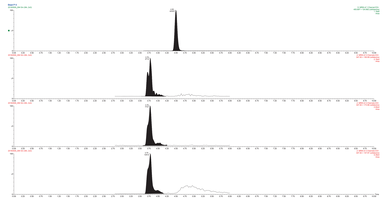
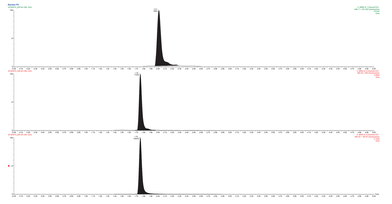
### Confirmatory LC-MS/MS chromatograms for RFM sample 5

#### Cephalexin



#### Kanamycin



## CONCLUSION

These findings indicate that the use of the IPM as a multi-analytical tool revolutionises the screening capacity for drug residues in test settings, which is beneficial for consumer protection. Sample P5 tested positive for tobramycin due to high concentration of kanamycin (4xMRL). Tobramycin was proven to be absent by LC-MS/MS.