

NEW BIOCHIP ARRAY FOR THE SIMULTANEOUS SCREENING OF IN –EXCESS OF 100 DRUG RESIDUES IN MILK COVERING EU REGULATED DRUGS

Tohill, A., Mahoney, J., Porter, J., McConnell, R.I., FitzGerald, S.P.

Randex Food Diagnostics, 55 Diamond Road, Crumlin, CoAntrim BT29 4QY, UK

INTRODUCTION

Monitoring the presence of veterinary drugs in milk is important for consumer protection. Currently milk screening tests do not assess all residues required under current EU legislation. This study reports the development of a new biochip array for the rapid and simultaneous qualitative detection of 98% of all EU

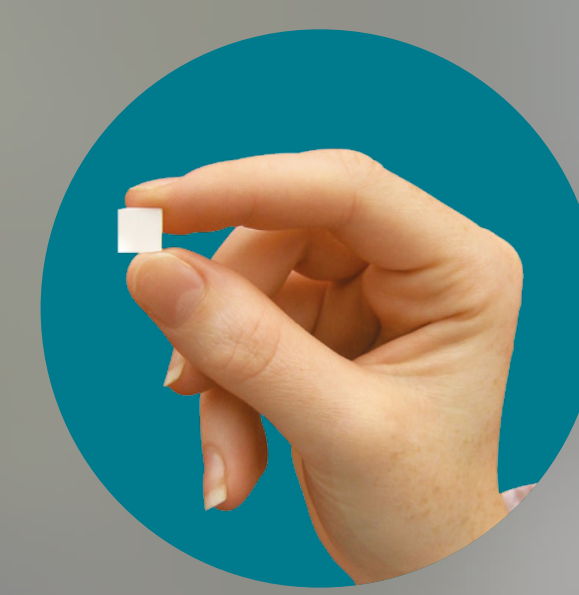
regulated anti-infectious/antibiotics and 80% of all EU regulated anti-inflammatory agents/nonsteroidal anti-inflammatory agents (EU Commission Regulation 37/2010) from a single sample of raw milk at or below regulatory limits.

METHODOLOGY

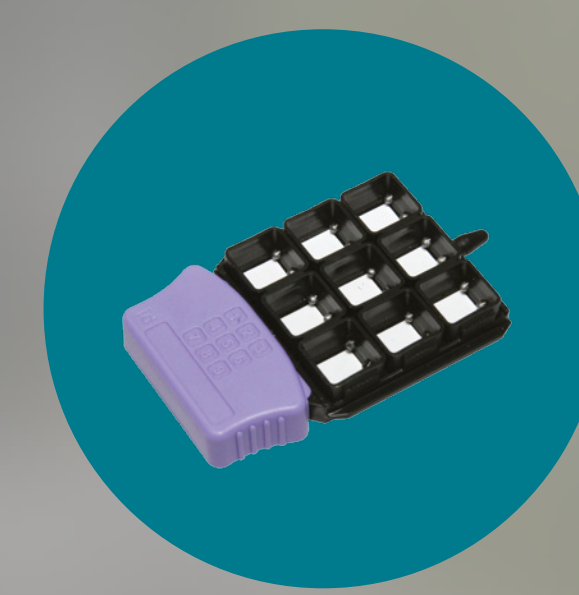
The InfiniPlex for Milk kit was used (EV4076, Randex Food Diagnostics, Crumlin, UK) Simultaneous competitive chemiluminescent immunoassays, defining discrete test sites on the biochip surface (9mm x 9mm) were applied to the semi-automated analyser Evidence Investigator (EV3602, Randex Food Diagnostics, Crumlin, UK).

The simultaneous immunoassays were applied to the semi-automated analyser Evidence Investigator (EV3602, Randex Food Diagnostics, Crumlin, UK).

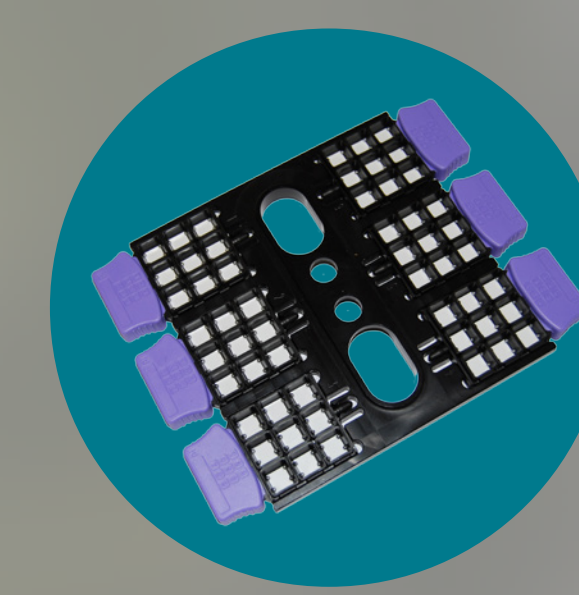
Raw milk samples (25µl) were added directly to the biochips. 48 samples can be assessed simultaneously within 2 hours.



Biochip
(9mm x 9mm)



Biochip carrier
(3 x 3 biochips)



Biochip handling tray
(6 biochip carriers = 54 biochips)



Semi-automated analyser
Evidence Investigator

RESULTS

Sensitivity profile of the biochip based immunoassays

Drug Residue	Decision Level (ppb) (≥ 95% positive results)	EU MRLs (ppb) ^{a,d}
Penicillin G	1	4
Ampicillin	2	4
Dicloxacillin	1	30
Amoxicillin	2	4
Cloxacillin	3	30
Oxacillin	2	30
Nafcillin	7	30
Cephalonium	0.3	20
Cefoperazone	1.5	50
Cephapirin	2.5	60
Cefquinome	12	20
Ceftiofur	30	100
Cefacetil	15	125
Cefazolin	22	50
Enrofloxacin	6.5	100
Ciprofloxacin	11	100
Danofloxacin	10	30
Tetracycline	13	100
Oxytetracycline	7	100
Doxycycline	23	NFU ^c
5-OH Flunixin	1	40
Meloxicam	9	15
Spiramycin	2	200
Spiramycin I	2	200
Tylosin A	32	50
Tilmicosin	34	50
Lincomycin	5	150
Pirlimycin	5	100
Hygromycin B	52	-
Polymixin B	0.15	-
Virginamycin MI	1	-
Flunixin	0.7	-

Drug Residue	Decision Level (ppb) (≥ 95% positive results)	EU MRLs (ppb) ^{a,d}
Metamizole (Dipyrone)	7	50
Tolfenamic acid	27	50
Phenylbutazone	1	5 ^b
Oxyphenbutazone	1	5 ^b
Chlormadinone	0.4	2.5
Dexamethasone	0.2	0.3
Betamethasone	1	0.3
Prednisolone	3	6
Methylprednisolone	0.5	2
Sulphaguanidine Sulphamethazine	7	100
Sulphadimidine	1	100
Sulphapyridine	1	100
Sulphadoxine	2	100
Difloxacin	20	NFU ^c
Marbofloxacin	9	75
Oxolinic Acid	12	NFU
Flumequine	22	50
Cephalexin	13	100
Erythromycin	1.6	40
Gamithromycin	8	NFU ^c
Tulathromycin	40	NFU ^c
Neomycin	11	1500
Paromomycin	2	NFU ^c
Streptomycin	23	200
Dihydrostreptomycin	14	200
Gentamicin	11	100
Kanamycin A	3	150
Novobiocin	4	50
Tobramycin	4	-
Bacitracin	2	100
Chlortetracycline	10	100
Cefuroxime	7	-

Drug Residue	Decision Level (ppb) (>95% positive results)	EU MRLs (ppb) ^{a,d}
Spectinomycin	3	200
Apramycin	5	NFU ^c
Chloramphenicol	0.2	<0.3 ^d
Florphenicol	0.7	NFU ^c
Thiamphenicol	2	50
Trimethoprim	0.7	50
Baqueloprim	8	30
Sulphamethoxazole	2	100
Sulphamonomethoxine	2	100
Sulphaquinoxaline	7	100
Sulphadimethoxine	3	100
Sulphachloropyridazine	4	100
Sulphadiazine	9	100
Sulphacetamide	4	100
Sulphamethoxyypyridazine	35	100
Sulfisoxazole	2	100
Sulphamerazine	21	100
Sulphamethizole	12	100
Sulphathiazole	10	100
Sulphabenzamide	1	100
Sulphameter	4	100
Sulphamoxol	34	100
Sulphanitran	5	100
Sulphaphenazole	3	100
Sulphatroxazole	2	100
Sulfisomidine	11	100
Dapsone	1	5 ^b
Nitroxylnil	0.4	20
Aflatoxin MI	0.03	0.05
Rifaximin	2	60
Colistin	2	50
Ractopamine	0.3	-

Examples of intra-assay precision data of biochip-based immunoassays

Assay	Mean Concentration (ppb)	Batch 1 CV (%)	Batch 2 CV (%)
Sulphonamide	10	5	7
Novobiocin	12.5	7	10
Erythromycin	20	4	6
Tetracyclines	25	8	7
Streptomycin	62.5	9	6
Phenylbutazone	5	10	6
Nitroxylnil	2	9	10
Hygromycin B	500	8	6
Tobramycin	30	8	9
Prednisolone	6	8	9
Bacitracin	10	9	9
Tolfenamic Acid	50	10	9
Aflatoxin MI	0.05	10	6

15.490.641

Matrix: Milk

^a Source: Annex III of Council Directive 2010.37/EC (amending Council Regulation 2377/90/EEC).
^b Source: CRL Guidance Paper (7 December 2007)
^c Not for use (NFU)
^d Minimum required performance limit (MRPL)

CONCLUSION

Features of InfiniPlex for Milk Kit:

- Qualitative screening of in excess 100 regulated drug residues from a single sample of raw milk without the need of sample preparation.
- Decision limits for the residues detected are well below MRLs and tolerance levels established by regulatory authorities.

- The application of the assay to the semi-automated biochip analyser Evidence Investigator allows the handling of 54 samples at a time.
- This represents a useful solution for the comprehensive screening of drug residues in milk and provides an analytical tool to detect and monitor residues/trends that cannot be currently tested to ensure milk quality is of a high standard. Samples giving positive test results using this assay must be further analysed with a confirmatory method.