

## APPLICATION OF EVIDENCE BIOCHIP ARRAYS TO THE MULTIPLEX SCREENING OF RESIDUES IN MILK

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### Introduction

Biochip array technology provides a platform for the simultaneous determination of multiple analytes with a single sample at a single point in time.

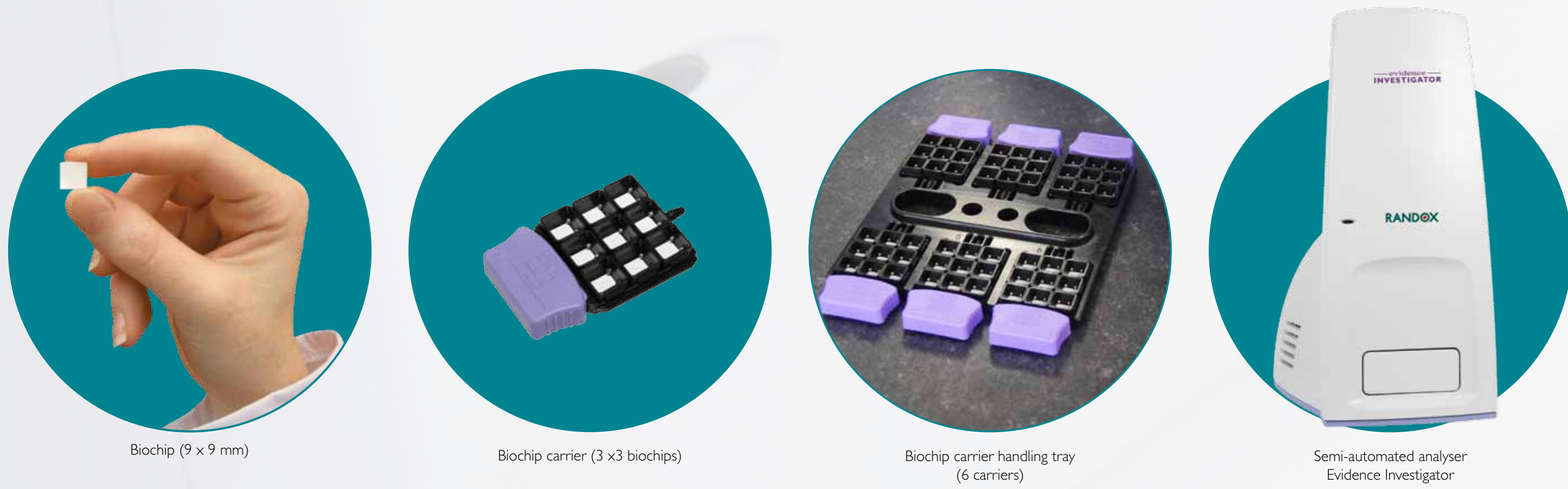
This multi-analytical approach increases the result output and is advantageous for the rapid and simultaneous screening of multiple residues in milk.

### Methodology

The biochip is the platform in which the capture ligands are immobilised and stabilised defining arrays of discrete test sites and it is also the vessel where the simultaneous immunoreactions are performed.

The biochips are supplied in a carrier (3x3 biochips per carrier), which are placed in the handling tray supplied. The handling tray has the capacity to accommodate six carriers (54 biochips).

The immunoassays for multi-analyte determination of residues in milk are applicable to the semi-automated bench top analyser Evidence Investigator (EV3602). The system incorporates dedicated software to process and analyse the multiple data generated.



Biochip (9 x 9 mm)

Biochip carrier (3 x3 biochips)

Biochip carrier handling tray (6 carriers)

Semi-automated analyser Evidence Investigator

#### Test menu

##### Anti-Microbial Array I Plus

Sulphadimethoxine  
Sulphadiazine  
Sulphadiazine  
Sulphamethazole  
Sulphamethoxazole  
Sulphamethoxypridazine  
Sulphachloropyridazine  
Sulphamerazine

Sulphamonomethoxine  
Sulphisoxazole  
Sulphathiazole  
Sulphamethazine  
Sulphaquinoxaline  
Sulphapyridine  
Trimethoprim

Anti-Microbial Array I Plus (EV3775)

##### Beta Lactam Array

Amoxicillin  
Amoxicillin  
Cloxacillin  
Dicloxacillin  
Nafcillin  
Oxacillin  
Penicillin G  
Penicillin V

Cefacetril  
Cefazolin  
Cefoperazone  
Cefquinome  
Cefuroxime  
Cephalexin  
Cephalonium  
Cephapirin

Beta Lactam Array (EV3793A)

##### Anti-Microbial Array II

Ceftiofur  
Florphenicol/Thiamphenicol  
Quinolones (generic)

Streptomycin/Dihydrostreptomycin  
Tetracyclines (generic)  
Tylosin/Tilmicosin

Anti-Microbial Array II (EV3524)

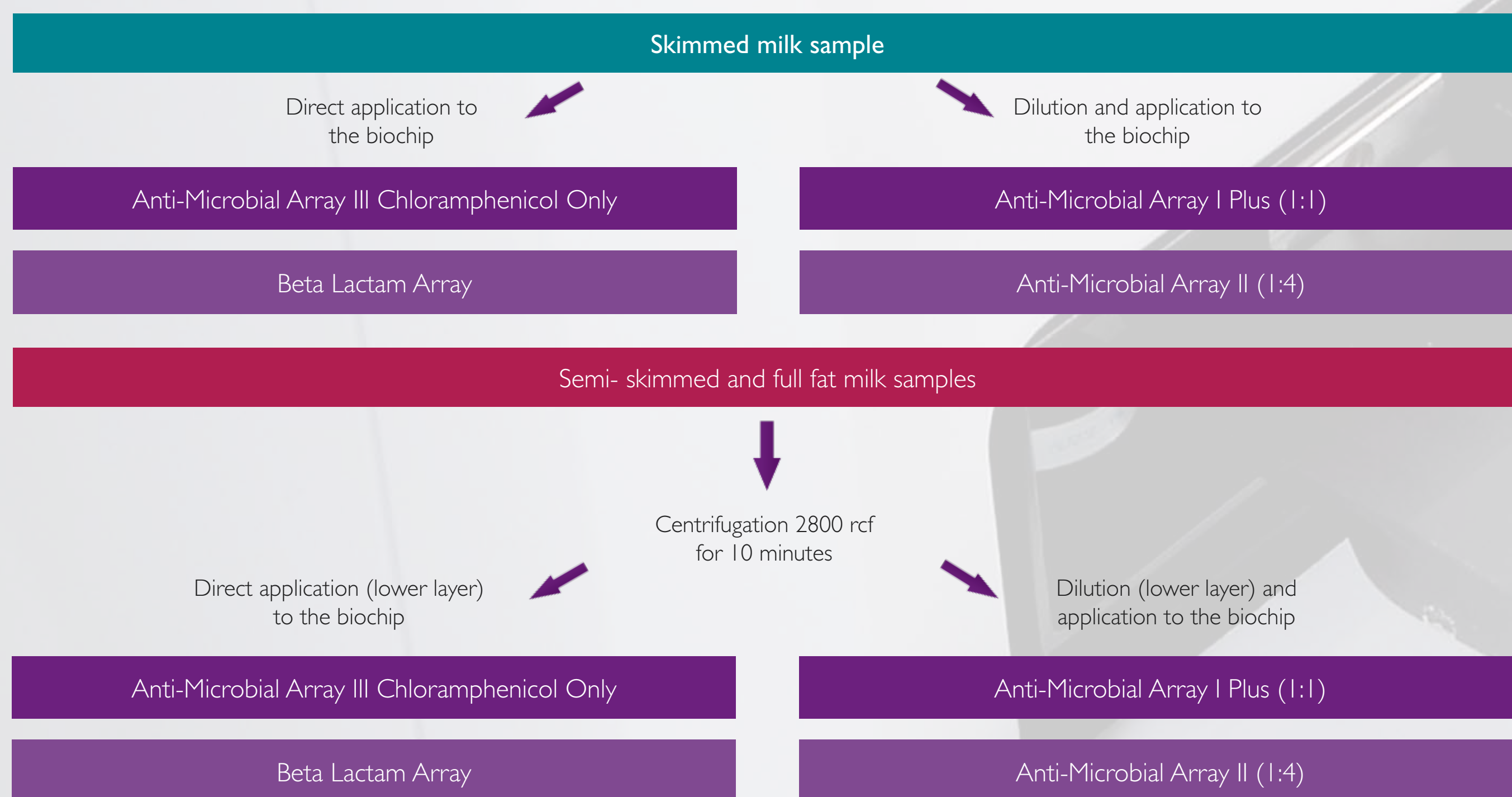
##### Anti-Microbial Array III Chloramphenicol Only

Chloramphenicol

Chloramphenicol Glucuronide

Anti-Microbial Array III Chloramphenicol Only (EV3739)

#### Sample preparation



### Results

#### Anti-Microbial Array I Plus

##### Limit of detection (LOD)

Assay	LOD (ppb)
Sulphadimethoxine	0.6
Sulphadiazine	0.5
Sulphadiazine	0.5
Sulphamethazole	0.5
Sulphachloropyridazine	0.5
Sulphamethoxazole	0.5
Sulphamethoxypridazine	0.5
Sulphamonomethoxine	2.0
Sulphamerazine	0.5
Sulphisoxazole	0.5
Sulphathiazole	0.5
Sulphaquinoxaline	2.5
Sulphapyridine	0.5
Trimethoprim	0.5

10623, 425, 435, 43795, 11048, 6495F

##### Specificity

- Fifteen simultaneous immunoassays were specific for the targets.
- The sulphamethoxypridazine assay presented 56% cross-reactivity with sulphaethoxypridazine.
- The sulphamethoxazole assay presented 92% cross-reactivity with sulphamethazole.

106249F

##### Precision

Typical intra-assay precision (n=20) <14% for different concentration levels.

106119F, 110559F

#### Anti-Microbial Array II

##### Limit of detection (LOD) & specificity/cross-reactivity

Assay	Compound	%Cross-reactivity	LOD (ppb)	Assay	Compound	%Cross-reactivity	LOD (ppb)
Quinolones	Norfloxacin	100	1	Ceftiofur	Ceftiofur	100	1.5
	Pefloxacin	84			Florphenicol	100	0.5
	Enrofloxacin	76		Thiamphenicol	53	0.5	
	Ciprofloxacin	59		Streptomycin	Streptomycin	100	2.0
	Ofloxacin	57			Dihydrostreptomycin	183	
	Enoxacin	54		Tylosin	Tylosin	100	2.5
	Pipemidic acid	36			Tilmicosin	37	
	Fleroxacin	32		Tetracyclines	Tetracycline	100	1.0
	Levofloxacin	32			4-epitetracycline	87	
	Nadifloxacin	27			Rolitetracline	67	
Orbifloxacin	23		4-epioxitetracycline		52		
Danofloxacin	20		Oxytetracycline		52		
Marbofloxacin	16		Chlortetracycline		51		
Oxolonic acid	12		Demeclocycline		41		
Difloxacin	8		Doxycycline		23		
Pazufloxacin	7		4-epichlortetracycline		20		
Sarafloxacin	6		Methacycline		11		

As per FU EV3524 Rev 310712zw

##### Precision

Typical intra-assay precision (n=20) <8% for different concentration levels.

As per FU EV3524 Rev 310712zw

#### Beta Lactam Array

##### Limit of detection (LOD)

Assay	LOD (ppb)
Amoxicillin	0.6

11053, 055, 603

##### Specificity

Beta-lactam antibiotic	Concentration (ppb) (>95% positive test results)	% Cross-reactivity
Amoxicillin	0.8	100
Amoxicillin	0.8	59
Cefacetril	12.5	7
Cefazolin	10	6
Cefoperazone	5	105
Cefquinome	2	12
Ceftiofur	20	11
Cephalonium	100	1
Cephalotaxin	2	237
Cephapirin	6	81
Cloxacillin	3	52
Dicloxacillin	3	70
Nafcillin	18	6
Oxacillin	3	47
Penicillin G	0.4	388
Penicillin V	0.4	246

As per FU EV3793 Rev20512bm

##### Precision

Intra Assay precision was determined by assaying 20 replicates of each sample. The intra-assay precision was determined to be ≤ 10%.

As per FU EV3793 Rev20512bm

#### Anti-Microbial Array III Chloramphenicol Only

##### Limit of detection (LOD)

Assay	LOD (ppb)
Chloramphenicol	0.04

As per FU EV3738 Rev310712zw

##### Specificity

Compound	%Cross-reactivity
Chloramphenicol	100
Chloramphenicol glucuronide	75.1

As per FU EV3738 Rev310712zw

##### Precision

Intra Assay Precision was determined by assaying 20 replicates of each of three levels of spiked control. The intra-assay precision was determined to be ≤ 10%.

As per FU EV3738 Rev310712zw

### Conclusions

- This multi-analytical approach to the screening of residues in milk consolidates many tests using a single platform and enhances the scope of tests and the test result output
- Simple sample preparation
- The system incorporates dedicated software to process and archive the multiple data generated

- With the semi-automated Evidence Investigator analyser 54 biochips can be handled at a time. Due to the generic nature of the antibodies used in this platform, the number of possible test results is increased (Anti-Microbial Array I Plus 765 test results, Anti-Microbial Array II 1530 test results, Beta Lactam Array 720 test results)