

CONFfIDENCE

Work package 2a Coccidiostats

May, 2012

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Outline

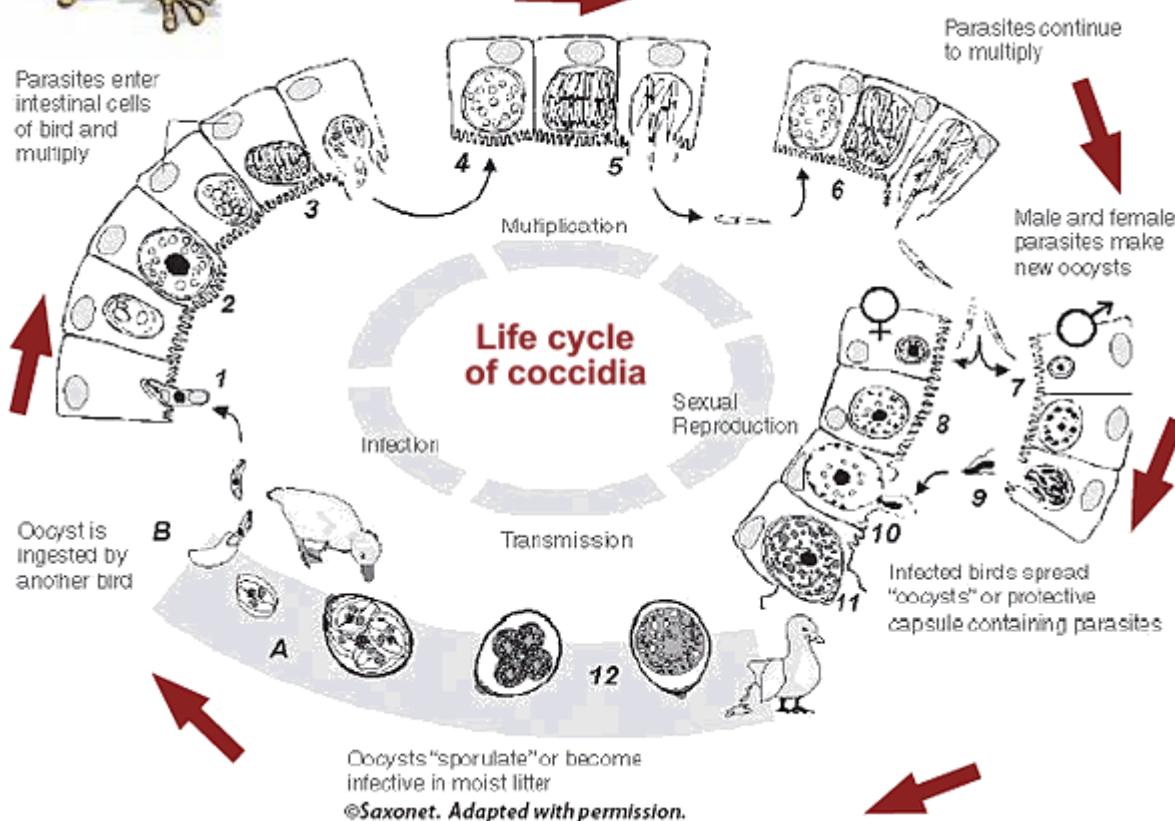
- Introduction
 - Coccidiostats/coccidiiodosis
 - Technology
 - Measurement
- Five-plex immunoassay in matrix
- Single-laboratory validation egg and feed
- Conclusions

Introduction

Coccidiosis



- Reduced growth
- Reduced egg production
- Increased mortalities

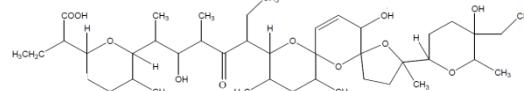




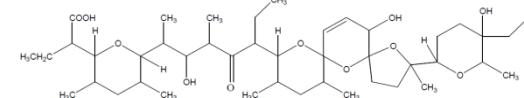
Introduction

Coccidiostatica

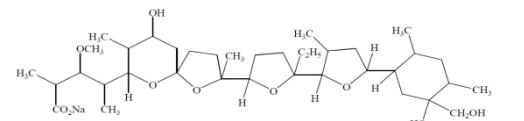
Salinomycin
Narasin
Monensin
Lasalocid
Nicarbazin
Diclazuril
Semduramicin
Maduramicin
Decoquinate
Halofuginone
Robenidine



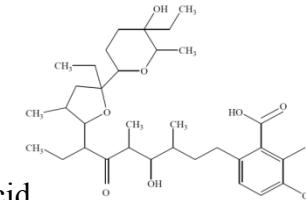
Salinomycin



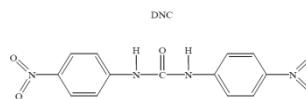
Narasin



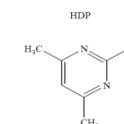
Monensin



Lasalocid

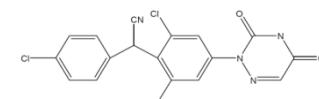


DNC



HDP

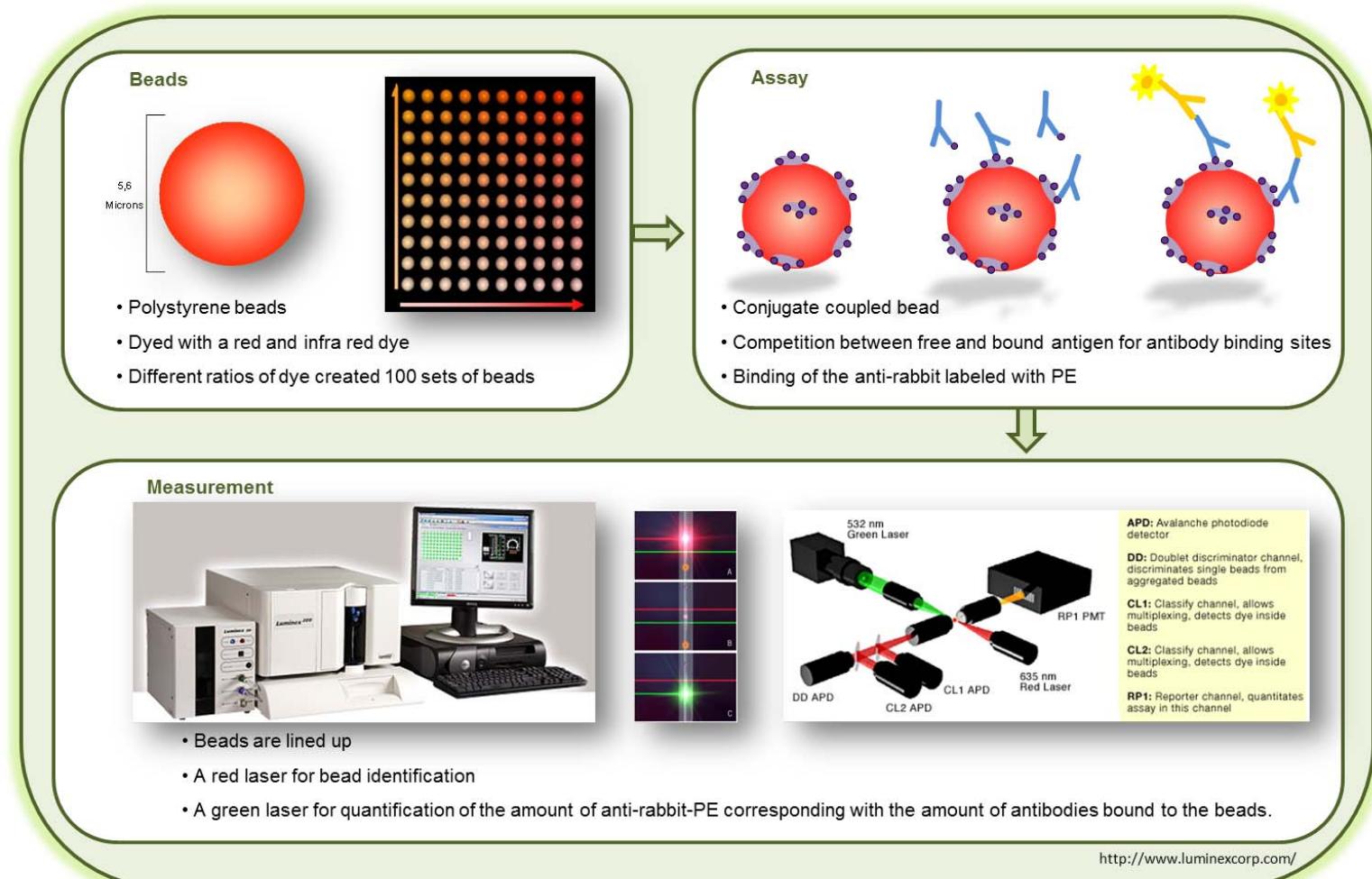
Nicarbazin



Diclazuril

Introduction

Technology

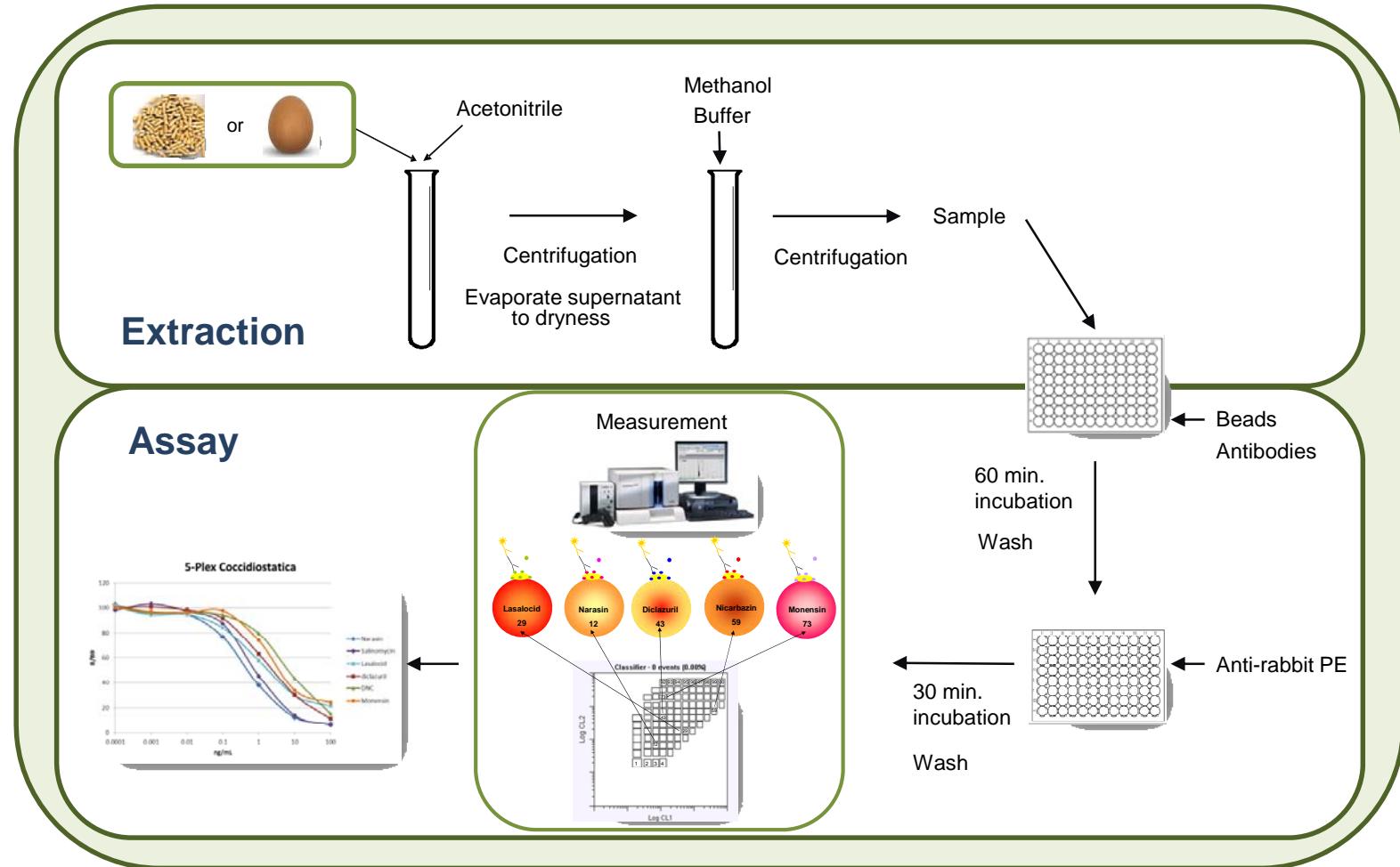


<http://www.luminexcorp.com/>

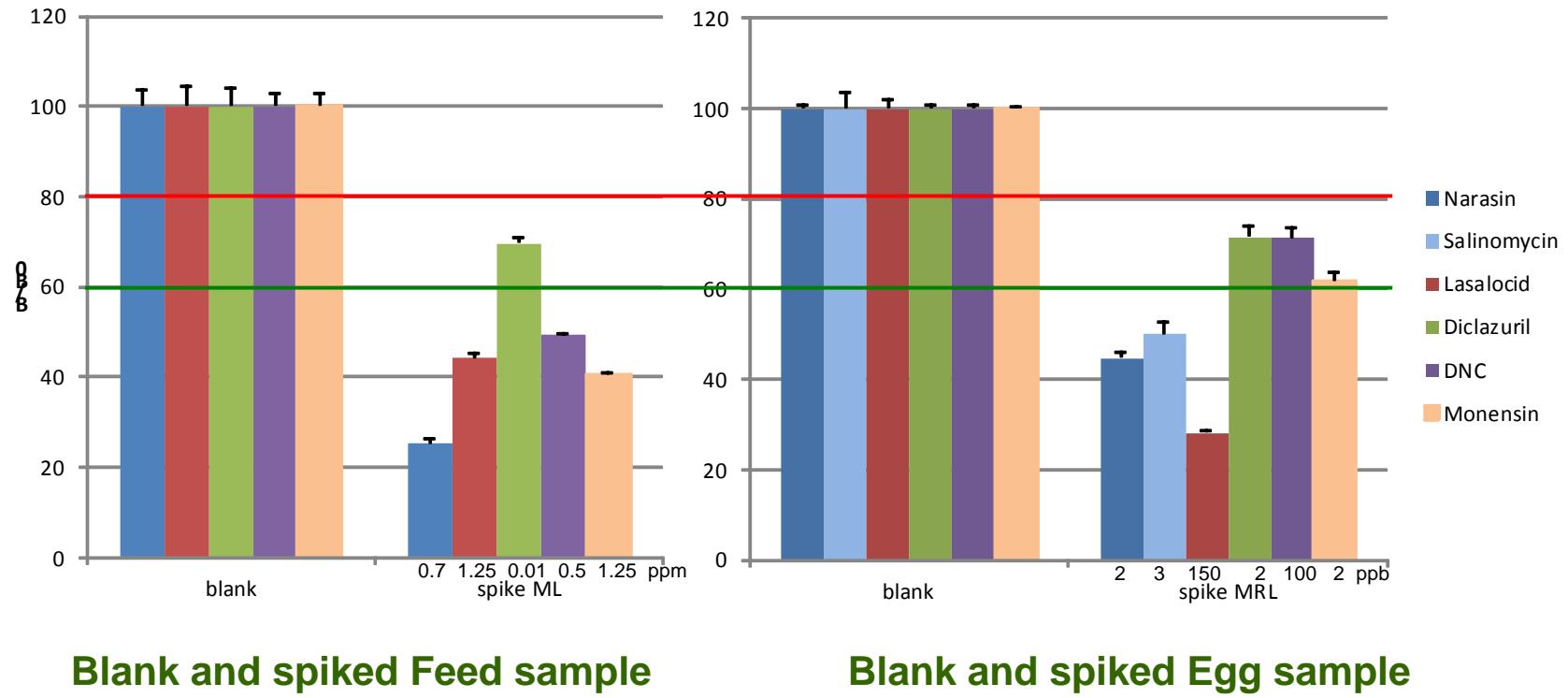


Five-plex immunoassay

Measurement



Five-plex immunoassay matrix



Single-laboratory validation

Validation of a screening method

Key requirement for validation of a screening method:
Prevent false compliant results

- 2002/657/EC: β -error < 5% at the level of interest
- New CRL guideline paper: $CC\beta < M(R)L$
 - Select compounds for validation
 - Define screening target concentration
 - Define cut off level
 - Initial validation (originator laboratory)
 - Abridged validation (receptor laboratory)
 - Continuous verification



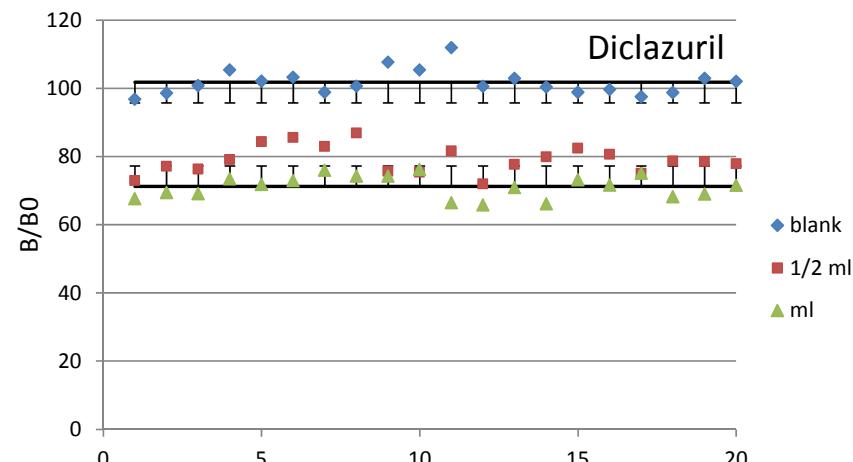
Single-laboratory Validation

Egg matrix



- 20 Different blank egg samples
- Spiked at 1 and 2 ng/g
(½ ML and ML level)
- Measurement on 3 days

Diclazuril	
lowest blank	94.3
highest 1/2 ML	86.8 cut-off level
CCb	1
ML	2



Single-laboratory Validation

Egg matrix



Egg	Narasin	Lasalocid	Diclazuril	DNC	Monensin	Salinomycin
Lowest blank	94.2	92.2	96.8	95.6	95.6	95.3
Highest 1/2 ML (cuf-off level)	59.3	39.4	86.8	88.9	81.8	78.0
CCb	1	75	1	50	1	2
ML	2	150	2	100	2	3
T	95.4	94.9	95.7	96.8	95.0	95.4
Mean of the blank (B)	101.2	100.6	101.8	101.0	100.5	101.2
std blank	3.6	3.4	3.7	2.5	3.4	3.6
Fm	49.2	32.9	77.2	78.5	73.5	57.9
Mean of the spike	43.3	27.2	71.2	74.3	68.0	53.5
Std spike	3.6	3.4	3.7	2.5	3.4	2.7
Fm < B	Yes	Yes	Yes	Yes	Yes	Yes
Fm < T	Yes	Yes	Yes	Yes	Yes	Yes
Intra assay CV% (20 samples)	5.2	11.3	4.5	3.9	7.2	4.8
Inter assay CV% (7 samples)	4.5	9.5	4.2	3.5	6.8	4.3
Intra assay CV% (1 sample)	1.4	3.0	2.3	1.4	1.8	1.4
Inter assay CV% (1 sample)	2.3	3.3	3.0	2.4	3.4	2.3

Single-laboratory Validation

Egg matrix



	Blank					
	Narasin	Salinomycin	Lasalocid	Diclazuril	DNC	Monensin
Variability due to the day in %	0	0	0	10	0	8
Variability due to the replicate in %	100	100	100	90	100	92
Relative standard deviation of measurements %	3.61	3.61	3.44	3.68	2.52	3.38
Rate of false positive %	0.000000	0.000000	0.000000	0.000135	0.000008	0.002909
	1/4 MRL					
	Narasin	Salinomycin	Lasalocid	Diclazuril	DNC	Monensin
Variability due to the day in %	0	43	58	46	0	44
Variability due to the replicate in %	100	57	42	54	100	56
Relative standard deviation of measurements %	6.35	7.12	12.22	9.72	6.42	6.61
Rate of false positive %	0.001	0.269	20.653	27.765	2.576	35.532
	1/2 MRL					
	Narasin	Salinomycin	Lasalocid	Diclazuril	DNC	Monensin
Variability due to the day in %	27	7	17	0	0	0
Variability due to the replicate in %	73	93	83	100	100	100
Relative standard deviation of measurements %	5.51	5.93	17.35	5.35	4.49	5.48
Rate of false positive %	2.857	2.766	82.938	33.558	24.805	95.095
	MRL					
	Narasin	Salinomycin	Lasalocid	Diclazuril	DNC	Monensin
Variability due to the day in %	9	53	6	0	0	0
Variability due to the replicate in %	91	47	94	100	100	100
Relative standard deviation of measurements %	6.99	5.46	13.82	4.86	4.81	12.84
Cut off %	48.6	58.6	33.7	77.2	80.5	83.1



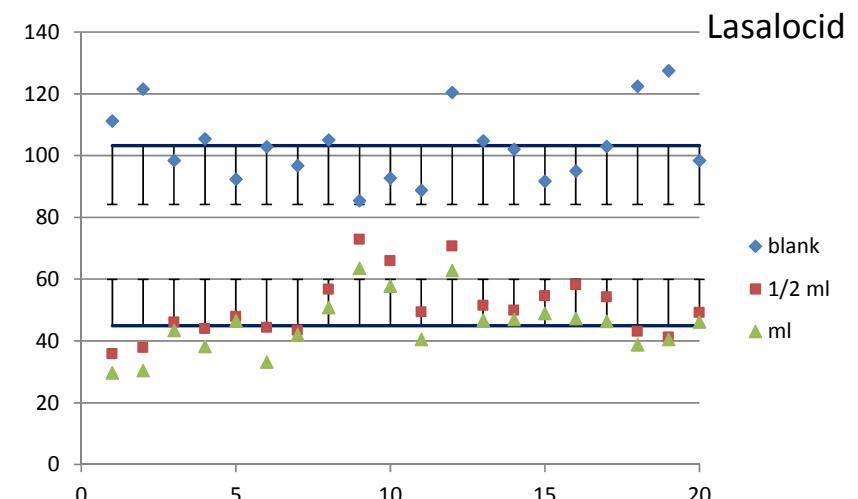
Single-laboratory Validation

Feed matrix



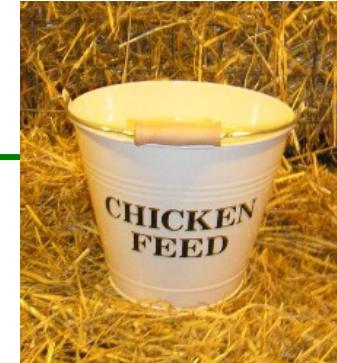
- 20 Different blank feed samples
- Spiked at 75 and 150 ng/g
(½ ML and ML level)
- Measurement on 3 days

Lasalocid	
lowest blank	85.3
highest 1/2 ML	72.9 cut-off level
CCb	75
ML	150



Single-laboratory Validation

Feed matrix



Feed	Narasin	Lasalocid	Diclazuril	DNC	Monensin	Salinomycin
Lowest blank	92.3	85.3	97.4	94.3	87.7	92.3
Highest 1/2 ML (cuf-off level)	71.8	72.9	108.7	79.7	80.1	82.2
CCb	1	75	1	625	1	2
ML	2	150	2	1250	2	3
T	86.0	83.6	93.0	93.3	90.8	86.0
Mean of the blank (B)	103.8	103.2	104.2	104.7	106.9	103.8
std blank	10.9	11.9	6.8	7.0	9.8	10.9
Fm	65.7	60.1	111.7	72.3	69.4	56.1
Mean of the spike	41.7	45.0	92.9	58.4	47.6	33.3
Std spike	14.6	9.2	11.5	8.4	13.3	13.9
Fm < B	Yes	Yes	No	Yes	Yes	Yes
Fm < T	Yes	Yes	No	Yes	Yes	Yes
Intra assay CV% (20 samples)	23.5	17.2	8.2	10.4	20.4	30.4
Inter assay CV% (7 samples)	23.9	13.5	8.3	10.3	19.9	30.4
Intra assay CV% (1 sample)	1.7	2.5	1.7	2.2	4.4	1.7
Inter assay CV% (1 sample)	0.3	3.4	1.3	2.2	2.8	0.3

Single-laboratory Validation

Feed matrix

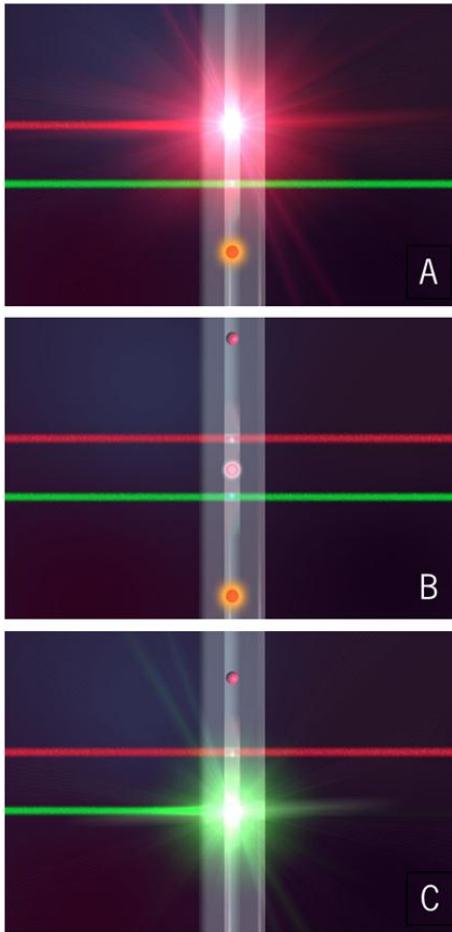


	Blank					
	Narasin	Salinomycin	Lasalocid	Diclazuril	DNC	Monensin
Variability due to the day in %	11.94	11.94	3.26	18.63	29.16	17.54
Variability due to the replicate in %	88.06	88.06	96.74	81.37	70.84	82.46
Relative standard deviation of measurements %	11.09	11.09	12.01	7.03	7.29	10.05
Rate of false positive %	0.202167	0.027954	0.150669	88.728361	0.020117	0.097827
	1/2 MRL					
	Narasin	Salinomycin	Lasalocid	Diclazuril	DNC	Monensin
Variability due to the day in %	5.27	14.81	54.57	2.47	0	12.58
Variability due to the replicate in %	94.73	85.19	45.43	97.53	100	87.42
Relative standard deviation of measurements %	12.92	18.00	11.11	5.73	7.18	13.68
Rate of false positive %	98.977702	76.196340	84.472527	98.977702	67.469963	86.033516
	MRL					
	Narasin	Salinomycin	Lasalocid	Diclazuril	DNC	Monensin
Variability due to the day in %	0	0	53.47	6.75	0	0
Variability due to the replicate in %	100	100	46.53	93.25	100	100
Relative standard deviation of measurements %	14.99	14.24	10.10	11.64	8.69	13.41
Cut off %	67.60	57.96	62.43	112.98	73.47	70.79



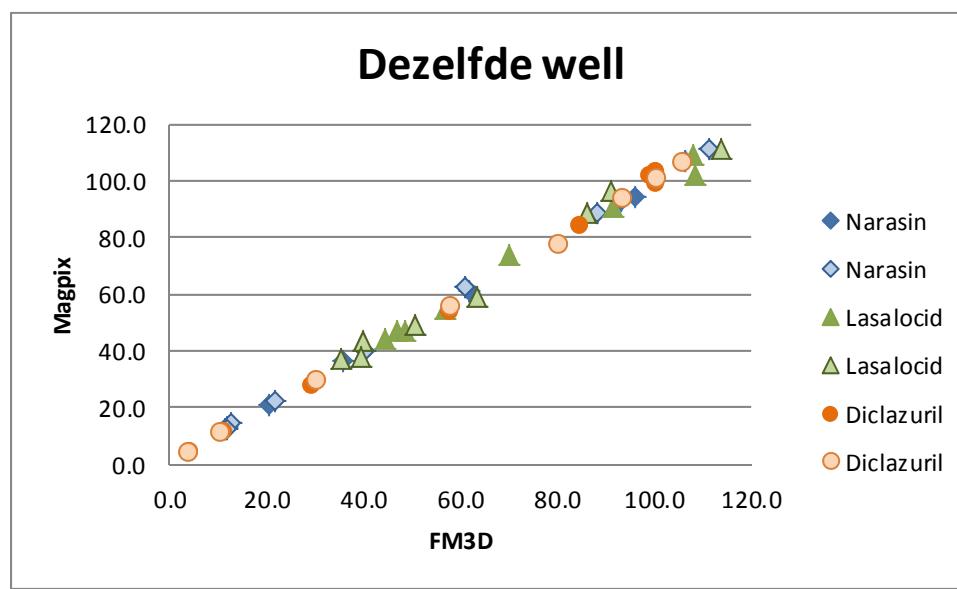
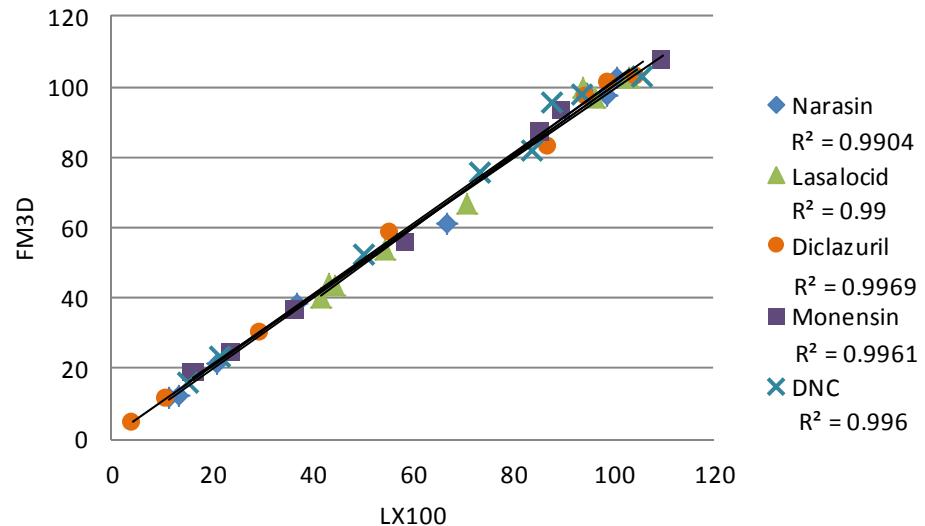
Instrument comparison

FM3D, LX100 and MagPix



Instrument comparison

FM3D, LX100 and MagPix



Conclusions

- Egg validation is completed
- Feed validation is completed
- Ring test is ongoing for egg and feed
- Publication:

Development of a five-plex flow cytometric immunoassay for the simultaneous detection of six coccidiostats in feed and eggs.

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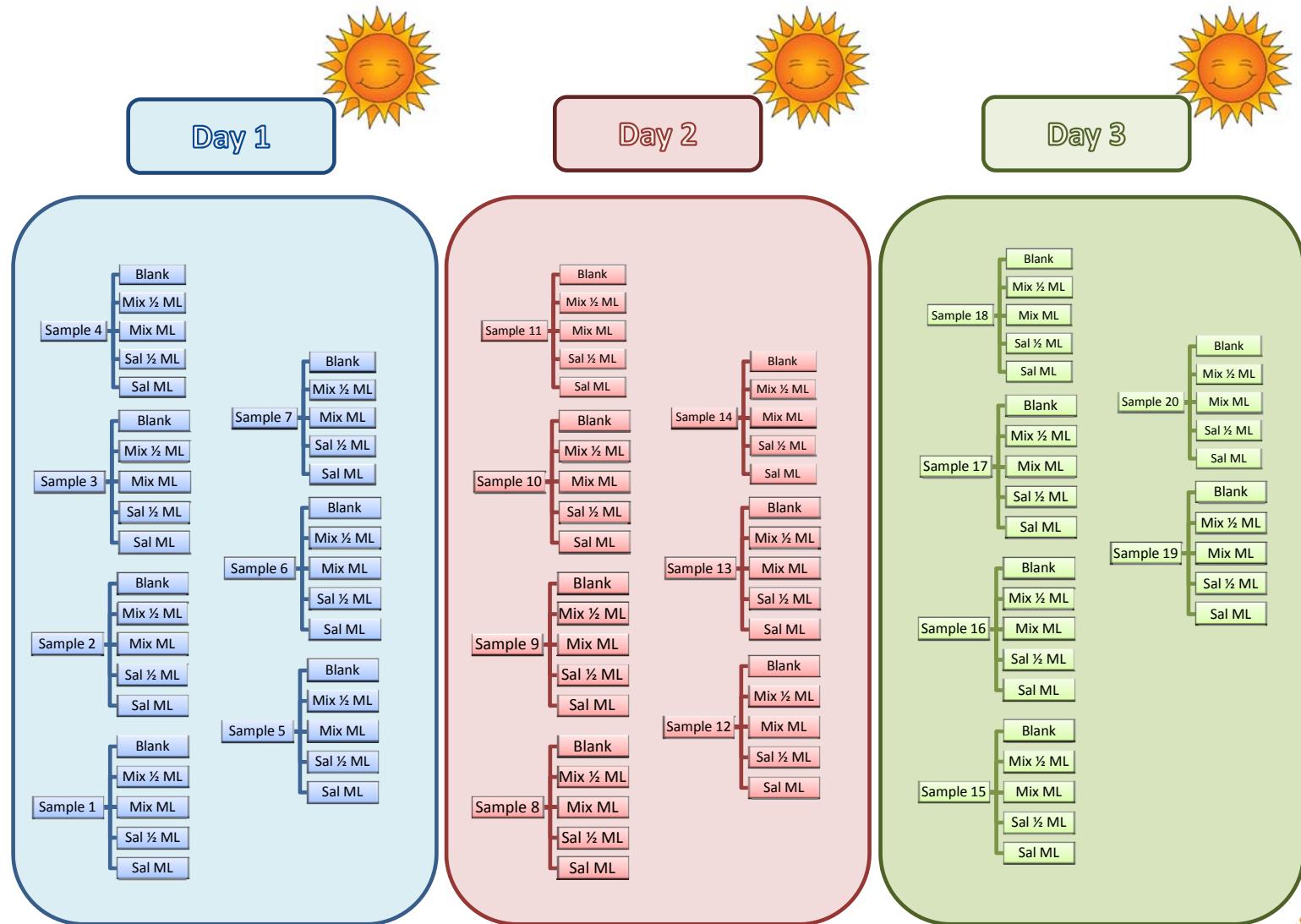


Thank you
for your attention



Single-laboratory validation

Validation scheme



Single-laboratory validation – Results

Eggs

- Major impact on the total variance: repeatability of the standard deviation
- Between day effect is minor
- ⇒ Robustness of the method
- Intermediate precision: less than 10% (5%) RSD - up to 17%
- ⇒ overall performance OK
- False positives for blanks: <1%
- ⇒ method fit for purpose of differentiating blanks from samples at or >MRL
- False positives for samples below MRL

