# Multiplex bead-based assay vor the on-site detection of 42 antimicrobials in animal drinking water and feather extracts

Monique E. Bienenmann-Ploum*, Bjorn J.A. Berendsen, Mariel G. Pikkemaat, Willem Haasnoot, Leendert A. van Ginkel and Toine F.H. Bovee

## Introduction

The increasing threat of antimicrobial resistance resulted in restrictive measures applied to the prescription and use of antibiotics. Monitoring is increased and rapid on-site screening methods to identify abuse of antimicrobials can support (governmental) inspections.
An on-site bead-based screening assay is developed for a rapid, sensitive and multiplex detection of antimicrobials belonging to the groups of sulfonamides, β-lactams aminoglycosides, tetracyclines and (fluoro)quinolones in animal drinking water and feather extracts.

## Method

Fifty different colour-coded bead sets are available for the MAGPIX® and can be chemically coupled with different antigens. This assay uses 7 bead sets (coated with an antimicrobial competitor) of which 6 combined and with antibodies and one with a receptor. All developed assays start with a competition between the antimicrobial and a competitor. The MAGPIX® measures the mean fluorescent intensity on the beads which correspond with the amount of bound bioreagents and is inversely related to the antibiotic concentrations in the sample (Figure 1).

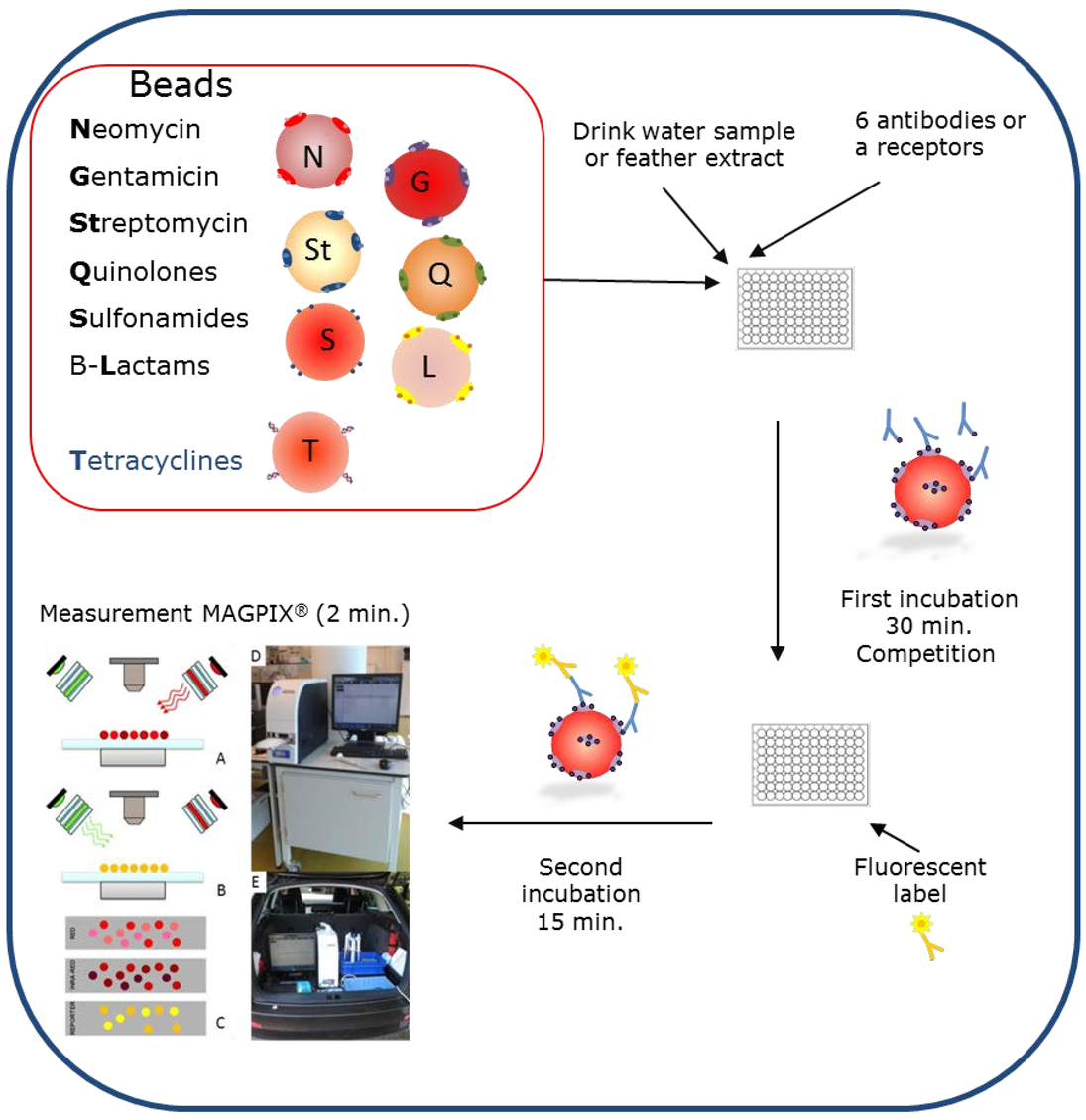


**Figure 1.** Overview of the general principle and procedural steps of the bead-based multiplex assay. MAGPIX® measurement: (A+B) beads are trapped with a magnet and identified by red led excitations and green led excitation for quantification of the bound fluorescent label. (C) The accomplishing MAGPIX® emission plots. (D) Laboratory and (E) on-site measurements

## Results

The detection capacity of the method was tested at 100 ng/mL in drinking water (Table 1). Forty-two antimicrobials showed inhibition of the maximum signal and could be detected at this level.

**Table 1.** Antimicrobials tested in animal drinking water at 100 ng/ml level

| | | Detection at 100 ng/ml | | Detection at 100 ng/ml |
|---|---|---|---|---|
| Aminoglycoside | Streptomycin | √ | Kanamycin | - |
| | Dihydrostreptomycin | √ | Apramycin | - |
| | Neomycin | √ | Paromomycin | - |
| | Gentamicin | √ | Spectinomycin | - |
| Quinolonen | Enrofloxacin | √ | Sarafloxacin | √ |
| | Flumequine | √ | Marbofloxacin | √ |
| | Ciprofloxacin | √ | Danofloxacin | √ |
| | Norfloxacin | √ | Oxolinic Acid | √ |
| | Difloxacin | √ | | |
| Tetracyclines | Tetracycline | √ | 4-Epi-tetracycline | √ |
| | Doxycycline | √ | 4-Epi-chlortetracycline | √ |
| | Oxytetracycline | √ | 4-Epi-oxytetracycline | √ |
| | Chlortetracycline | √ | | |
| Sulfonamides | Sulfadimidine | √ | Sulfadimethoxine | √ |
| | Sulfamethoxazole | √ | Sulfamethoxypyridazine | √ |
| | Sulfachloropyridazine | √ | Sulfapyridine | √ |
| | Sulfaquinoxaline | √ | Sulfamerazine | √ |
| | Sulfaoxazole | √ | Sulfadoxine | √ |
| | Solfatroxazole | √ | Sulfamonomethoxine | √ |
| | Sulfathiazole | √ | Sulfamethiazol | √ |
| β-Lactam | Ampicillin | √ | Cloxacillin | √ |
| | Amoxicillin | √ | Oxacillin | √ |
| | Penicillin G | √ | Nafcilline | √ |
| | Penicillin V | √ | Cefquinome | - |
| | Dicloxacillin | √ | | |
| Others | Trimethoprim | - | | |
| | Tylosine | - | | |
| | Chloramphenicol | - | | |

An on-site pilot experiment with feathers (extracts) and animal drinking water samples showed promising results. Even retrospective detection was possible as the treatment with amoxicillin could still be detected in feathers even after a week.
An initial validation with drinking water samples has been performed. The assay will be fully validated for enforcement and monitoring purposes.

## Conclusions

- We developed a multiplex bead-based assay for the detection of at least 42 antimicrobials below 100 ng/mL level in animal drinking water, comprising aminoglycosides, sulfonamides, tetracyclines, (fluoro)quinolones and β-lactams.
- The assay can be applied for feather extracts.
- This assay can be easily extended with other assays, e.g. for the detection of other antimicrobials or growth promoters, e.g. β-agonists.

## Acknowledgements

Unisensor (Liege, Belgium) for suppling us with all tetracycline assay components, the NVWA (Wageningen, The Netherlands) for suppling us with on-site sample materials. Aart van Amerongen of Wageningen UR Food & Biobased Research (Wageningen, The Netherlands) and Ministry of Economic affairs for their financial support.

RIKILT Wageningen UR
P.O. Box 230, 6700 AE Wageningen
Contact: monique.bienenmann@wur.nl
T + 31 (0)317 480256
www.wageningenUR.nl/en/rikilt

## References

Bovee, T.F.H. *et al*. (2016) Food Additives and Contaminants, accepted for publication.
de Keizer, W., Bienenmann-Ploum, M. E., Bergwerff, A. A., & Haasnoot, W. (2008). Analytica Chimica Acta, 620(1-2), 142-149.
Haasnoot, W., de Pré, J., Cazemier, G., Kemmers-Voncken, R., Verheijen, R., Janssen, B.J.M., (2000) Food Agric. Immunol. 12.
Haasnoot, W. *et al*. (2002) Residues in Milk Food and Agricultural Immunology Vol 14(1), pages 15-27
Adrian, j. *et al*. (2009) TrAC-Trend. Anal. Chem. 28, 769-777.