The ABC assay: a rapid screening technique for measuring antibiotic activity in environmental samples

A. J. Smith, A. Raffo and J. L. Balaam
Centre for Environment, Fisheries & Aquaculture Science, Burnham on Crouch
www.cefas.co.uk

Aim

The aim of this research was to establish a screening method for identifying and measuring antibiotic activity in the environment. Such activity could arise both from antibiotic parent compounds, and also from the products of their metabolism.

Photograph by Adam Hart-Davis

Introduction

- Antibiotics are prescribed to both the human and farm animal populations of many countries in both Europe and the rest of the world.
- Up to 50% of the antimicrobial use in humans is considered inappropriate (Paskovaty *et al*, 2005),
- Antibiotics are finding their way back into the environment.
- This may impact the rate at which organic matter in the environment is broken down, or the effectiveness of sewage treatment works.
- At low concentrations, antibiotics can lead to the development of antibiotic- resistant strains of the microbes responsible for disease in humans (Guillemot, *et al.*, 1998, Yoneyama, and Katsumata, 2006, Drlica, 2003).
- Resistance in microbes is currently only associated with hospitals and similar institutions.
- The identification of new environmental niches creating 'superbugs' is likely to be a cause for concern.
- Similar research in environmental settings is limited
- Compounds other than the parent chemical may be actively antibiotic.

Method

Premitest

Premitest (DSM food industries, Netherlands), is commercially available in kit form and has been shown to be responsive to all antibiotics tested with it (42 in number, Stead *et al.*, 2004). It provides a simple yes/no response to presence of antibiotics. using *Bacillus stearothermophilus*, a thermophilic bacteria, which is responsive to all of the most commonly used antibiotics and provides a measurable effect when exposed to samples of contaminated meat or other substrate. We aimed to adapt this method to enable the testing of environmental samples, developing an antibiotic challenge (ABC) assay.

Colour development in sample vials after incubation

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Results

Direct application

Early testing was carried out using standards directly applied to the vials. Two antibiotics were tested using this methodology at a range of dilutions to produce simple dose-response curves. Erythromycin was chosen as one of the antibiotic 'standards' because it is known to be present in detectable amounts in environmental samples (Roberts and Thomas, 2006). Sulfamethoxazole was selected as a standard as a representative of the totally man-made antibiotics. A selection of representative anthropogenic contaminants (Triclosan, Zinc and Diuron) were also screened to establish whether they could cause false positives. False positives were found, but at environmentally unrealistic concentrations.

Table 1: ABC assay results from directly applied spiked stocks. The number in brackets indicates the number of hours incubation

Chemical	NOEC (hours)	LOEC (hours)
Sulphamethoxazole	440 µg/l(3)	132.5 µg/l(3)
Erythromycin	11 µg/l (3) 50 µg/l (3.5)	23 µg/l (3) 110 µg/l (3.5)
Zinc	5,800 µg/l(3)	10,000µg/l(3)
Diuron	33,000 µg/l(3) 100,000 µg/l (4)	56,000 µg/l(3) -
Triclosan (Irgasan)	7,000 µg/l (3) 10,000 µg/l (3.5) 15,000 µg/l (4)	10,000 µg/l (3) 15,000 µg/l (3.5) 20,000 µg/l (4)
H20, Lab Lemco	All	-

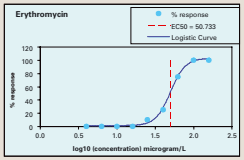
Extracted spiked samples

Antibiotic spiked samples were made up in deionised water, extracted and eluted with methanol and transferred into nutrient medium at a concentration factor of 1000:1.

The antibiotics, erythromycin and sulfamethoxazole, were tested at a range of concentrations, and in simple binary mixtures. Zinc and 4,4'-DDT have also been tested as anthropogenic toxicants, but were not found to be active at all.

Table 2: ABC assay results from extracted spiked stocks. The number in brackets indicates the number of hours incubation.

Chemical	NOEC	LOEC
Sulphamethoxazole	- 0.25 µg/l (4.3)	0.1 µg/l (3) 0.8 µg/l (4.3)
Erythromycin	-	0.25 µg/l (4.3)
SM/ EM Mix	-	0.25 µg/l (4.3)
Zinc	15,000 mg/l (3)	-
4,4'-DDT	100,000 mg/l (3.5)	-
H20, Lab Lemco	All tests	-



Erythromycin dose-response curve

Mixed effluent entering an estuary

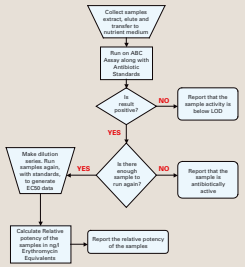
Environmental samples

A selection of environmental samples were analysed. Samples that showed strong activity were made into a dilution series and re-analysed including an erythromycin dilution series for comparison. The end point for the assay is defined as the time at which the blanks turned yellow. All other vials were read for colour at the same time. Vial colour at the end of each test was recorded on the five-point scale in Table 3.

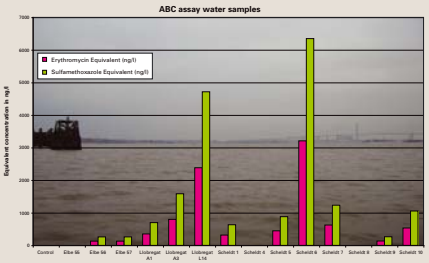
Results for the water and sediment samples can be seen in the two charts.

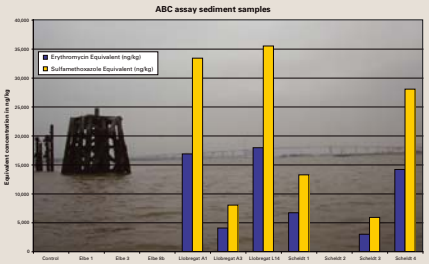
Table 3: ABC assay colour response with antibiotic standards for comparison

Colour	Yellow	Yellow with some purple	50/50	Purple with some Yellow	Purple
Response	0%	25%	50%	75%	1000%
Comparative Erythromycin Potency	<25 µg/l	40 µg/l	50.7 µg/l	63 µg/l	>100 µg/l
Comparative Sulfamethoxazole Potency	<40 µg/l	40 µg/l	100 µg/l	155 µg/l	>250 µg/l



Method flow chart





Discussion

This assay is intended as a screen for the rapid assessment of sites to establish whether there is antibiotic activity present and at what relative levels of activity. Once key sites have been identified in this way directed chemical analysis can be used in a cost effective way to identify the exact antibiotics present. The screen is rapid, inexpensive and capable of a very high throughput. Results are easy to interpret and can even be read in the field.

The assay utilises living bacteria, which means that it also responds to the presence of any antibiotically active compounds formed during the metabolic breakdown of parent chemicals. This gives a measure of total antibiotic activity in an environmental sample, including compounds that may not be identified using directed chemical analysis.

Conclusions

1. Although still a new technique with further validation to carry out, the ABC assay shows promise as a tool for the assessment of antibiotic activity in the environment.
2. The assay works as a rapid, inexpensive yes/no test for antibiotic activity in environmental samples.
3. False positives can be avoided by incorporating cytotoxicity data from other bioassays and by further research into assay behaviour in the presence of non-antibiotic contaminants.
4. Toxic metals which could lead to false positives are not retained during the water sample extraction process, reducing the false positive response.
5. More statistically useful responses, including EC50s and antibiotic equivalents can be determined by using a 'score' for the colour change.
6. Directly applied standards, extracted water and sediment samples can all be assessed using this assay.

The 96-well plate method is on its way…

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andy.smith@cefas.co.uk