

CARDIOTOX ASSAY

Biobide is a biotechnological company offering drug discovery services to Pharma, Biotech, Chemical, Cosmetic and Nutraceutical companies. Our service is based on the **zebrafish model** and the capacity to offer highly efficient tailor made assays.

Zebrafish models are gaining recognition in their applications within several fields, such as developmental biology and toxicology.

The advantages of the zebrafish are mainly their low cost and ease of maintenance and breeding. Moreover, the zebrafish is ideal for research purposes due to its small size, ease of handling and transparency. In fact, integrating the use of fluorescent reporter genes into this model allows the visualization of specific tissues, such as the heart. Despite clear anatomic differences between the zebrafish two-chambered (one atrium and one ventricle) heart and four-chambered mammalian heart, several studies have highlighted similarities in the genes and regulatory networks driving cell fate.

Importantly, the use of zebrafish larvae is in accordance with the 3R principle.

The assay to evaluate cardiotoxicity is performed under Good Laboratory Practice (GLP) environment.

METHOD DESCRIPTION

Experimental model: zebrafish (*Danio rerio*) strain expressing Green Fluorescent Protein (Figure 1) obtained from crossing adult zebrafish under strict environmental conditions of temperature and photoperiod.

Treatment: 48 hpf (hours post fertilization) embryos are placed in 96-well plates, one embryo/well, 20 embryos/condition and 5 concentrations per compound. Embryos treated with terfenadine at 5µM as positive control are included. DMSO at 0.5% is used as vehicle.

Assessment: After 3 and 24 hours of exposure the embryos are analyzed (Figure 2).

Imaging: A 15 second video of the beating heart is recorded and analyzed using non-commercial Cardio v3.0.0.5 software (Biobide property) to obtain the heart rate, the presence of arrhythmia and the absence of heartbeat (death/fibrillation). Embryos are not anesthetized to avoid potential beating alterations.

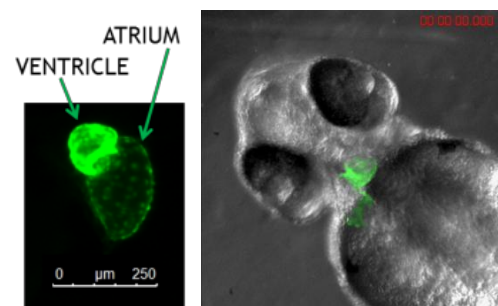


Figure 1. Zebrafish strain expressing Cop-GFP

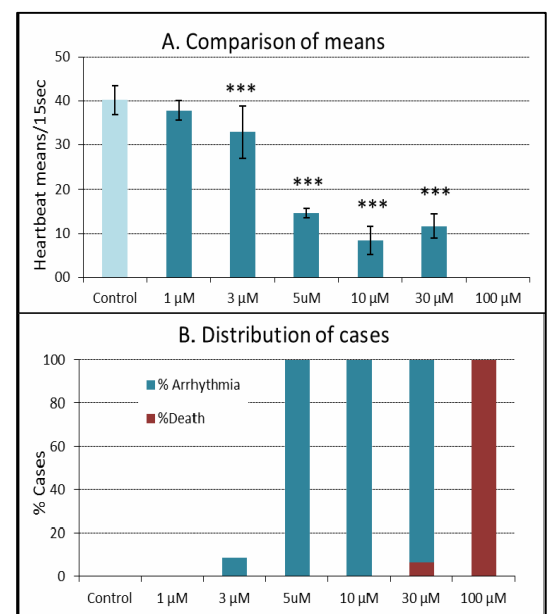


Figure 2. Example of graphs showing the results of embryos treated for 3 hours with terfenadine. (A) Bar graphs representing mean and standard deviation of the number of heartbeats counted in 15 seconds; (B) Percentage of embryos displaying arrhythmia and dead embryos; p>0.05; ** p>0.01; *** p>0.001.

VALIDATION RESULTS

Biobide's Validated Cardiotoxicity Assay comparing expected Cardiotoxic effects in humans with detected Cardiotoxic effects in zebrafish in Biobide (Table 1).

Table 1. List of drugs with different human therapeutic indications that were tested and results obtained.

Classification of products	Reference Compounds	Cardiac effect in humans	Cardiotox assay	Classification
Antiarrhythmics	Lidocaine	Bradycardia	Bradycardia	TP
	Flecainide	QT prolong.	Arrhythmia 2:1	TP
	Propafenone	QT prolong.	Arrhythmia 2:1	TP
Beta-blockers	Amiodarone	QT prolong.	Arrhythmia 2:1	TP
	Propranolol	Bradycardia	Bradycardia	TP
Antipsychotics	Timolol	Bradycardia	Bradycardia	TP
	Thioridazine	QT prolong.	Arrhythmia 2:1	TP
	Haloperidol	QT prolong.	Arrhythmia 2:1	TP
	Ziprasidone	QT prolong.	Bradycardia	FN
	Pimozide	QT prolong.	Arrhythmia 2:1	TP
	Sertindol	QT prolong.	Arrhythmia 2:1	TP
Antidepressants	Risperidone	QT prolong.	Arrhythmia 2:1	TP
Antitumorals	Fluoxetine	Arrhythmia	Arrhythmia	TP
Calcium blockers	Tamoxifen	Arrhythmia	Arrhythmia	TP
	Lapatinib	No effect	No effect	TN
	Verapamil	Bradycardia	Bradycardia	TP
	Diltiazem	Bradycardia	Bradycardia	TP
	Nitrendipine	Bardycardia/ Cardiac arrest	Bardycardia/ Cardiac arrest	TP
Colinergic agonist	Terodiline	QT prolong.	Arrhythmia 2:1	TP
	Pilocarpine	No effect	No effect	TN
Antihistaminics	Nicotine	Bradycardia	Bradycardia	TP
	Astemizol	QT prolong.	Arrhythmia 2:1	TP
	Terfenadine	QT prolong.	Arrhythmia 2:1	TP
Anti-infective	Fexofenadine	No effect	No effect	TN
	Halofantrine	QT prolong.	Arrhythmia 2:1	TP
Gastrointestinal agents	Foscarnet	Bradycardia	Bradycardia	TP
	Cisapride	QT prolong.	Arrhythmia 2:1	TP
Hormones	Estradiol	Bradycardia	Bradycardia	TP
	Testosterone	Bradycardia	Bradycardia	TP
Iks blockers	L-768763	QT prolong.	Bradycardia	FN
Ina Openers	SDZ-201106	QT prolong.	Arrhythmia 2:1	TP
Others	Digitoxin	Bradycardia	Cardiac arrest	TP
	Ketanserin	Bradycardia	Bradycardia	TP

TP: true positive

TN: true negative

FP: false positive

FN: false negative

High sensitivity: 100%
High specificity: 93.3%

FURTHER ANALYSIS CAN BE PERFORMED:

- Bioavailability by HPLC MS/MS to investigate further false negative results.
- Histopathology.
- Gen expression.

✓ Zebrafish cardiotoxicity study is an alternative, rapid and non-invasive assay with good sensitivity (100%) and specificity (93.3%) that is suitable to be used to early evaluate cardiotoxicity.

✓ Zebrafish embryos can be used in drug discovery to provide early screening assays which comply with 3R principle.