

Report on the drug screening survey organized by the COST B22 Working group 3 "Drug Evaluation"



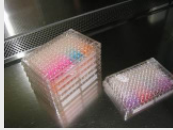
Reto Brun, Swiss Tropical Institute, Basel, Switzerland
 Donatella Taramelli, Department of Public Health- Microbiology- Virology University of Milan, Italy

Introduction

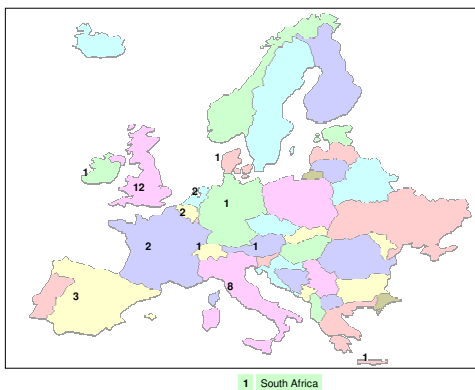
Working Group 3 of the COST Action B22 is called 'Drug Evaluation'. The objectives of this WG3 were defined as follows:

- 1) To compile an inventory of labs and their expertise in drug screening and evaluation in vitro and in animal models
- 2) To offer screening and evaluation possibilities to scientists working on synthetic compounds or natural products
- 3) To improve and standardize drug screening methodologies

In an attempt to accomplish objective 1) a survey form was sent to all country representatives for distribution among scientists active in that field. The survey was asking for the name of the responsible scientist and institution, and for short description of in vitro assays and animal models for the different protozoan parasites which are established. This poster reports on the outcome of this survey.



WG3 Screening survey summary



36 report forms received

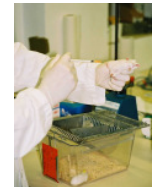


List of Groups Participating in the Survey on Screening Methods

- | | |
|--------------------------------------|--------------------------------------|
| Behnke J (Nottingham, UK) | Kharazmi A (Copenhagen, DK) |
| Bell A (Dublin, IE) | Krauth-Siegel L (Heidelberg, G) |
| Bolas FF (Madrid, ES) | Maes L (Antwerp, BE) |
| Bosisio E (Milan, IT) | Mazier D (Paris, FR) |
| Brandonisio O (Bari, IT) | McConkey G (Leeds, UK) |
| Brun R (Basel, CH) | Noeldl H (Vienna, AT) |
| Carriò J (Barcelona, ES) | Oppendoes (Brussels, BE) |
| Carter KC (Glasgow, UK) | Savoia, Daniela. (Torino, Italy) |
| Cioli D (Rome, IT) | Shallig H (Amsterdam, NL) |
| Coles G (Bristol, UK) | Scoulica E (Crete, GR) |
| Costi MP (Modena, IT) | Severini C (Rome, IT) |
| Egan T (Cape Town, SA) | Smith D (York, UK) |
| Fairlamb AH (Dundee, UK) | Taramelli D (Milan, IT) |
| Gonzalez-Pacanoska D (Granada, ES) | Tielens, Aloysius G.M. (Utrecht, NL) |
| Hablutzel, Annette (Camerino, Italy) | Townson S (Harrow, UK) |
| Haddad F (Bradford, UK) | Trees A (Liverpool, UK) |
| Harnett W (Glasgow, UK) | Vial H (Montpellier, FR) |
| | Wright C (Bradford, UK) |
| | Yardley V (London, UK) |

Parasite species and number of labs

	<i>P. falciparum</i>	19		<i>L. donovani</i>	6
	<i>P. berghei</i>	9		<i>L. major</i>	8
	<i>P. chabaudi</i>	3		<i>L. infantum</i>	4
	<i>P. vinckei</i>	2		<i>L. mexicana</i>	2
	<i>P. yoelii</i>	3		<i>Toxoplasma gondii</i>	2
	<i>T. cruzi</i>	7		<i>Babesia divergens</i>	1
	<i>T. brucei sp</i>	7		<i>GI nematodes</i>	1
	<i>S. mansoni</i>	2		<i>Trichinella spp</i>	1
	<i>Giardia intestinalis</i>	3		<i>Fasciola hepatica</i>	1
	<i>Brugia spp</i>	1			



Alamar Blue Assay

Bloodstream forms in axenic culture (*T. brucei* sp.)

in a 96-well plate

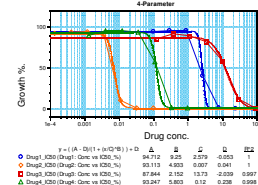
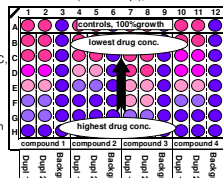
Serial drug dilutions

Incubation: 72h, 37°C, 5% CO₂

Add Alamar Blue

Fluorescent detection

Determine IC₅₀



In vitro Subcellular assays

<i>P. falciparum</i> only (7 labs)	Other protozoans (6 labs)
Plasmeprin II & IV	Lm dUTPase
Phospholipid metabolism	Lm DHFR-TS
Cyclophilin binding	Lm PTR1
Microtubulin inhibition	Lm N-myristoyl transferase
Aminopeptidase Inhibition	Tc dUTPase
FK506 binding-protein	Tc DHFR
Pf dUTPase	Tc PTR1
N-myristoyl transferase inhibition	Tb N-myristoyl transferase
Haemozoin formation	Carbohydrate metabolism
	Glycosyltransferases
	Choline pathway
	Sterol biosynthesis
	Human DHFR, dUTPase, TS

Methodologies employed for "multitarget" assays

Parasites	Assay	Method
<i>P. falciparum</i>	Sporozoites	Intrahepatic growth
	intraerythrocytic	³ Hypoxanthine, pLDH, Picogreen, HRPII
<i>Leishmania</i> spp	Promastigotes	Acid Phosphatase; Flow cytometry
	Axenic Amastigotes	Alamar blue (Resazurin)
	Amastigotes in macrophages	Giemsa staining; β -lactamase; Real time PCR
<i>T. brucei</i> spp	Trypomastigotes	Alamar blue (Resazurin)
<i>T. cruzi</i>	Amastigotes in MO, L-6 cells, MRC-5	β -galactosidase
<i>Giardia intestinalis</i>	Trophozoites	Alamar blue; microscopy
<i>Schistosoma mansoni</i>	Adult worm	Survival in culture
<i>Babesia divergens</i>	intraerythrocytic	In vitro growth

In vivo models

Species	labs	test
<i>Plasmodium</i> spp	8	4 day test in rodents, prophylaxis, hepatic stage
<i>P. berghei</i> – <i>Anopheles stephensi</i>	1	In vivo prophylactic activity
<i>Leishmania</i> spp	7	In vivo transmission blocking activity
<i>Leishmania</i> spp	7	cutaneous & visceral leishmaniasis
<i>T. cruzi</i>	3	Chagas disease mouse model
<i>T. brucei</i> sp	4	African trypanosomiasis, acute & chronic CNS model
<i>Schistosoma</i> spp	2	Survival test in rodents
<i>Brugia</i> spp	1	Survival test in rodents
<i>Fasciola hepatica</i>	1	Survival test in rodents
<i>GI nematodes</i>	1	Survival test in rodents

The list of labs and the survey form are available on the COST website: <http://www.icp.ucl.ac.be/cost/costB22/wg3.htm>

Send your reply to: donatella.taramelli@unimi.it