
	DEPARTMENT PARASITOLOGY BPRC Rijswijk	SOP: 001 Version: 001
	Rabbit malaria-immune Ig for Growth Inhibition Assay (GIA) standardisation	Page 1 of 2
		Date: 29/11/10

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A. Introduction

The BG98 GIA standard was raised in 100 rabbits at BioGenes, Berlin, Germany, 2 rabbits died during the immunisation phase. The standard is referred to as BG98 (BioGenes 98).

B. Description

This material was prepared from a serum pool derived from rabbits immunised with recombinant malaria vaccine candidate antigen Apical Membrane Antigen (AMA-1).

Rabbits were immunised on days 0, 28 and 56 with a mixture of 7 AMA1 alleles (FVO, 3D7, HB3, CAMP, DiCo1, DiCo2 and DiCo3) in a potent, non-disclosed, adjuvant. The total dose of AMA1 was 49µg (7µg of each of the 7 AMA1 proteins). Rabbits were exsanguinated on day 70 and the immunoglobulin fraction was purified from serum using ion-exchange chromatography. The immunoglobulin fraction was filter sterilised, exchanged into RPMI at a concentration of 30 mg/mL and subsequently lyophilised at National Institute for Biological Standards and Control (NIBSC) on behalf of the Biomedical Primate Research Centre (BPRC) for the standardisation of in-vitro growth inhibition assays for *Plasmodium falciparum*.

Each vial contains only purified rabbit immunoglobulin (Ig) and buffer (RPMI with traces of methyl red as indicator).

C. Contents

Each vial contains the residue of 0.47mL of a solution of 30mg/mL purified rabbit anti-malaria Ig in RPMI corresponding to a theoretical content of 14 mg Ig. The vials have been back-filled with N₂ at just below ambient pressure. The measured content by SEC HPLC is 13.39 ± 0.39mg. The preparation does **not** contain material of human origin. Vials can be stored at ambient temperature but for long-term storage -20°C is recommended.

D. Caution

This preparation contains material of rabbit origin. It is intended for use as a reference preparation in in-vitro bioassays. This preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures, such as the wearing of protective gloves. Care should be exercised in opening the vials in order to avoid cuts.

E. Reconstitution and use in GIA

Each vial should be reconstituted in 0.47mL sterile distilled water to yield a concentration of about 30mg/mL Ig in 1 x RPMI-1640. It is recommended to verify the concentration of the reconstituted BG98 by Spectrometry at 280 nm using the IgG molar extinction coefficient. Repeated measurements at BPRC have confirmed the 30mg/mL concentration of the reconstituted BG98 standard.

The final Ig concentration for the BG98 standard in GIA is 6 mg/mL. This 6 mg/ mL concentration typically yields 80-90% inhibition against laboratory isolates like FCR3, FVO, HB3, 3D7 and CAMP. In order to prepare a 2x stock for use in a GIA, the reconstituted BG98 (at 30 mg/mL) is further diluted to 12 mg/mL by the addition of 705 µL sterile RPMI-1640.

F. Storage

Reconstituted vials can be stored at ambient temperature but for long-term storage -20°C is recommended.