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Establishment of replacement International Standard 13/132 for human antibodies to *Toxoplasma gondii*

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ABSTRACT

Sixteen laboratories carried out a collaborative study to validate 13/132 as a replacement International Standard (IS) for TOXM (3rd IS for anti-Toxoplasma Serum, Human, 1000 IU). 13/132 is a freeze dried preparation of pooled human plasma from six donors who experienced a recent *Toxoplasma gondii* infection. The potency of 13/132 was compared to TOXM and 01/600 (1st IS for anti-Toxoplasma IgG, Human, 20 IU). Samples were tested for IgA, IgG, IgG avidity and IgM in agglutination assays; enzyme linked immunosorbent assays (ELISA), enzyme linked fluorescent assays, immunoblots, immunofluorescence assays and the Sabin–Feldman dye test for Ig. 13/132 was strongly positive for Ig, IgA, IgG and IgM and the reproducibility was very good. 13/132 contains high levels of anti-Toxoplasma Ig, IgG and IgM and its potency falls between TOXM and 01/600. The avidity of IgG was found to be low, similar to the avidity of IgG from TOXM. 13/132 was established by the Expert Committee on Biological Standardization as the 4th IS for Antibodies, Human, to *T. gondii* with an assigned unitage of 160 IU per ampoule for Ig by dye test and 263 U per ampoule for IgG by ELISA.

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1. Introduction

Toxoplasmosis is caused by the parasite *Toxoplasma gondii*. Congenital transmission of *T. gondii* remains a considerable burden on global health, with the highest incidence of 3.4/1000 births reported for South America [1]. The main objective of screening programmes is to prevent infection of the foetus by the parasite during pregnancy, and serology is widely used to diagnose Toxoplasmosis during pregnancy [2]. In addition, toxoplasmosis is a major cause of mortality among transplant patients [3]. The provision of appropriate antibody standards enables diagnostic laboratories and manufacturers of diagnostic tests to validate serologic assays to diagnose this infection. In 1994, Hansen et al. carried out a collaborative study to validate TOXM as a reference reagent for anti-Toxoplasma Ig by Sabin–Feldman dye test. Each ampoule of TOXM was assigned 1000 IU of anti-Toxoplasma Ig and subsequently established as the 3rd International Standard (IS) by the Expert Committee on Biological Standardization (ECBS) of the World Health Organization [4]. The dye test is a complement-mediated cell killing assay, utilising toxoplasma tachyzoites and does not distinguish between immunoglobulin classes that bind complement [5]. Although the assay is now carried out by fewer laboratories, the dye test is still considered a reference test and a confirmatory assay to validate commercial assays [6]. Therefore the dye test remains an important assay for the standardisation of anti-toxoplasma Ig levels in individuals suspected of toxoplasmosis. TOXM is used by manufacturers of *in vitro* diagnostic tests, national reference laboratories and hospital laboratories. Since 2000, stocks of TOXM have been low and these are now nearly exhausted. In 2003, 01/600 was established by ECBS as the 1st IS for anti-Toxoplasma IgG with a unitage of 20 IU per ampoule relative to TOXM [7,8]. 01/600 has a low level of IgG, which falls within the linear range of commercially available

Abbreviations: CV, coefficient of variation; ECBS, Expert Committee on Biological Standardization; ELFA, enzyme linked fluorescent assay; ELISA, enzyme linked immunosorbent assay; GCV, geometric coefficients of variation; GM, geometric mean; HSDA, high sensitivity direct agglutination assay; IFA, immunofluorescence assay; IS, International Standard; ISAGA, imunosorbent agglutination assay; kD, kilo Dalton; NIBSC, National Institute for Biological Standards and Control.

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Table 1
Characterisation of samples used in this study.

Study code	NIBSC code	Description of source material	Results of native material		Results of freeze dried material	
			Dye test[a] (IU mL^{-1})	IgM ELISA[b] (EU mL^{-1})	Dye test (IU mL^{-1})	IgM ELISA (EU mL^{-1})
TOXM	TOXM	3rd IS for anti-Toxoplasma Ig Human	–	–	1000[c]	–
A	01/600	1st IS for anti-Toxoplasma IgG Human	–	–	20[c]	–
B	01/576	Pool of seven normal human sera	–	–	<2[c]	–
C and E	13/132	Candidate IS for antibodies, human, to *T. gondii* from a pool of 6 plasma donations[d]	794 (500–1000)	101 ± 12	561 (500–1000)[ns]	98 ± 7[ns]
D	174	Anti-Toxoplasma plasma from one donor[e]	1000	105 ± 1	1000	125 ± 7
F	637	Anti-Toxoplasma plasma from one donor[e]	250	94 ± 6	500	101 ± 7

[ns]: Differences between native and freeze dried samples are not significant.
–: Not done.
[a] Lab code 6.1. Results given as geometric mean titre (range).
[b] Lab code 6.2. Results given as geometric mean ELISA Unit (EU) ± standard deviation: > 40: positive; >100: strongly positive (12).
[c] Taken from Rigsby et al., 2004 [8].
[d] Taken from 6 measurements over three days.
[e] Taken from 2 measurements over two days.

immunoassays used by diagnostic laboratories to distinguish between historic, background and diagnostic levels of IgG. Although, the unitage of 01/600 can be traced back to the 2nd IS TOXS, ECBS did not consider IS 01/600 a suitable replacement for TOXM because of the low levels of specific IgG and absence of specific IgM. The committee decided that a replacement for TOXM should contain high levels of IgM and IgG [4,7,8].

Recently, plasma samples from acute cases of Toxoplasmosis were acquired and a preliminary analysis showed that individual samples and the pooled sample 13/132, contained high levels of specific IgM and IgG, the latter with low to borderline avidity (see Table 1 and results not shown). Specific IgG of high avidity is seen as a marker of latent toxoplasmosis, whereas IgG of low avidity can be indicative of a recent infection [9,10]. A collaborative study was designed to validate 13/132 as an IS to replace TOXM. Participants were asked to test 13/132 in the dye test, and in addition were encouraged to use assays that are part of their diagnostic routine. The primary aims of the study were to:

1) assess the suitability of 13/132 as an IS for human anti-Toxoplasma Ig.
2) compare the reactivity of 13/132 relative to TOXM and 01/600 in the dye test.
3) compare the reactivity of 13/132 relative to TOXM and 01/600 in immunoassays for IgM and IgG, including avidity assays.
4) assess the reactivity of 13/132 in agglutination assays, immunoassays and in other titration assays currently in use.

2. Materials and methods

2.1. Participating laboratories and assay codification

Sixteen laboratories from 12 countries, including national reference laboratories, took part in the collaborative study. Details are given in the acknowledgement section. Throughout the study, participating laboratories were identified by a randomly assigned code number to maintain confidentiality. Data were collected and analysed at the National Institute for Biological Standards and Control (NIBSC). Each participant received two sets of seven samples comprising coded ampoules A to F including 01/600 (A) and duplicates of 13/132 (C and E), and one ampoule of TOXM (see Table 1).

2.2. Samples used in the study

Samples labelled A to F and TOXM were distributed as lyophilised preparations in duplicate sample packs by courier at room temperature. The samples were reconstituted following 'instructions for use' issued by NIBSC. Samples of 13/132 that were exposed to an elevated temperature range (−20 °C to +45 °C) to ascertain stability of the active component were distributed on dry ice. A brief characterisation of the samples, study codes, NIBSC codes and their reactivity in the dye test and the IgM capture enzyme linked immunosorbent assay (ELISA) are given in Table 1.

2.3. Characterisation of the proposed International Standard 13/132

Plasma samples were donated with informed consent by 6 female individuals of 21–33 years of age and obtained from Cerba Specimen Services (Saint-Ouen l'Aumône, France). At NIBSC, all samples tested negative for antibodies to Human Immunodeficiency Virus 1 and 2, Hepatitis C RNA and Hepatitis B surface antigen. Samples were stored at −80 °C until further use. Prior to pooling, samples were defrosted and stored at 2–8 °C overnight. The next day, samples were pooled (volume appr. 3 L) during which clotting occurred. Clots were removed by a filtration step using Whatman filter paper (1001-150). The filtrate of the pool was stored at 2–8 °C overnight and dispensed in 0.5 mL aliquots into glass ampoules coded 13/132 on the following day. The mean fill weight for 123 ampoules was 0.5156 g (CV of 0.16%). On the same day, freeze-drying under vacuum was started and completed after four days. Ampoules were back filled with pure N_2 and the mean O_2 content of 12 ampoules was 0.17% (CV of 53.71%). This implies ampoules passed the test for integrity, because the presence of cracks would be associated with an O_2 level of 21% similar to that found in the atmosphere. The mean residual moisture level in 12 ampoules was 0.6608% (CV of 18.89%). One hundred and sixty ampoules were rejected during the production process, 50 ampoules were held for accelerated degradation studies and 3695 ampoules were stored at −20 °C. These are available for distribution by NIBSC.

Native and freeze-dried samples of 13/132 and individual samples 174 (D) and 637 (F), which are part of the serum pool, were tested by dye test and IgM capture ELISA to determine the effect of freeze drying on specific Ig and IgM respectively (see Table 1). No significant differences in the mean values for levels of specific IgM and Ig were found before and after freeze-drying. Differences in unitage determined by dye test were found following freeze drying for samples C and E (13/132) and samples D and F. These fall within the four fold range and are therefore not considered significant.

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