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Kailin Yap

Tara Cawley

Phillip Matson  
*Edith Cowan University*

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## Internal quality control for the direct MAR IgG test: A simple and effective method using spiked seminal plasma

Kailin Yap<sup>1\*</sup>, Tara Cawley<sup>1</sup>, Phillip Matson<sup>1,2,3</sup><sup>1</sup>Fertility North, Joondalup, Western Australia<sup>2</sup>Edith Cowan University, Joondalup, Western Australia, Australia<sup>3</sup>Specialist Medical Centre, Joondalup Health Campus, Shenton Avenue, Joondalup 6027, Western Australia

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## ABSTRACT

**Objective:** To introduce a system using spiked seminal plasma for the QC of the MAR direct test, and to facilitate the determination of assay variability. **Methods:** A simple quality control (QC) system for use in the direct MAR test was developed using samples prepared by adding serum to antibody-negative centrifuged seminal plasma to obtain optimal binding, and storing 0.4 mL aliquots at  $-20^{\circ}\text{C}$  in straws. The serum was either from vasectomised men (positive control) or an antibody-negative woman (negative control). QC samples were thawed and mixed 1:1 with donor semen and pre-incubated for 1 hr at  $37^{\circ}\text{C}$ , and tested for sperm antibodies using the direct method of the MarScreen IgG kit (Fertility Technology Resources, USA). Two batches of controls were prepared and one of these was run on each day. **Results:** The negative controls invariably gave binding of less than 5%, whereas the two positive controls had binding (mean  $\pm$  sd) of  $89.5\% \pm 6.2\%$  (coefficient of variation [CV] = 7.0%) and  $(97.2 \pm 2.5)$  (CV = 2.6%). **Conclusions:** In summary, spiked seminal plasma incubated with whole semen can be used as a QC sample in the direct MAR IgG test.

### 1. Introduction

Sperm antibodies occur in approximately 5% of men attending an infertility clinic [1, 2], and have been associated with impaired spermatozoal function [3]. The mixed antiglobulin reaction (MAR) test was originally described as a screening tool [4] and has continued to be used widely [5, 6], with the SpermMar test using latex beads being popular [7]. Quality control (QC) can be done using pooled seminal plasma or serum [8], but the reality is that serum is used more frequently due to the volumes and the level of titre required [9]. Serum must be analysed using the indirect MAR test method [2], and so neat serum samples are an inappropriate QC of the direct method. The present report describes a system using spiked seminal plasma which can be (i) introduced simply in to a laboratory for the QC of the MAR direct test, (ii) stored frozen, and (iii) facilitate the determination of assay variability.

### 2. Materials and methods

Sperm antibodies were detected using the MarScreen IgG kit (Fertility Technology Resources, USA) [7] according to the manufacturer's instructions, but amended in line with the WHO recommendation by using  $3.5 \mu\text{L}$  volumes instead of  $10 \mu\text{L}$  [6]. Pools of antibody-free seminal plasma were mixed with antibody-positive serum from vasectomised men to make the positive controls, and female antibody-free serum to make the negative controls. QC samples were stored at  $-20^{\circ}\text{C}$  as 0.4 mL aliquots in straws. The spiked seminal plasma was thawed each day a MAR-test was performed and mixed 1:1 with antibody-free donor semen and incubated at  $37^{\circ}\text{C}$  for 1 hour. The donor semen was normozoospermic [6] to ensure sufficient motile sperm for the validity of the test. The resulting sample was then tested with the direct method after the mixing and pre-incubation stages described above.

### 3. Results

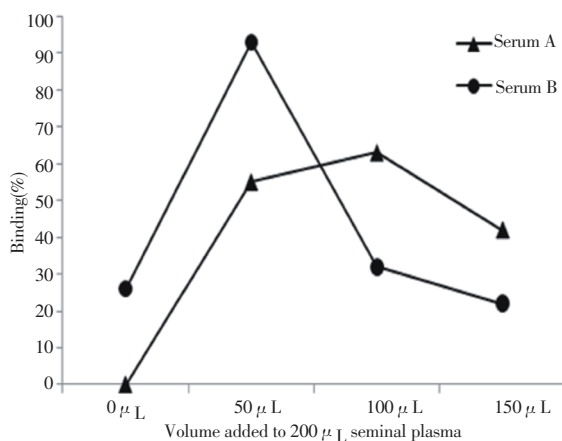
The two positive controls were made by initially adding

\*Corresponding author: Kailin Yap, Fertility North, Suite 213, Specialist medical Centre, Joondalup Health Campus, Shenton Avenue, Joondalup 6027, Western Australia, Australia.

E-mail: kailin@fertilitynorth.com.au

different amounts of antibody-positive serum to antibody-free seminal plasma as shown in Figure 1. It can be seen that there is an optimal dilution, and that the addition of serum to a higher concentration beyond the optimal level reduces the binding. Positive controls were therefore made with serum A by adding 7 mL serum to 14 mL seminal plasma, whilst 3.75 mL serum B was added to 15 mL seminal plasma. The associated negative controls were made by adding the same ratio of antibody-negative serum to the seminal plasma.

The two negative controls were included in daily runs and under routine conditions invariably gave binding <5%. The performance of the two positive controls is summarised in Table 1. They were both always positive confirming that the method worked on the day, and the values obtained showed coefficients of variation of 7% or less.



**Figure 1.** The binding of latex beads to sperm in the direct MAR test. Samples were made by adding 0–150 µL antibody-positive serum to 200 µL antibody-free seminal plasma. The resulting mixture was then added to an equal volume of normozoospermic donor semen, incubated at 37 °C for 1 hour, and tested with the MarScreen IgG kit.

**Table 1**

Assay variability.

	Positive sample	
	QC 1	QC 2
No. runs	46	20
Mean binding	89.5 ± 6.2	97.2 ± 2.5
Coefficient of variation	7.0%	2.6%

#### 4. Discussion

Agglutination between the beads serves as an internal positive control for the antibody-antigen reaction in the MAR test [6], but it is good practice to include positive and negative controls when sperm antibody tests are performed [10]. The World Health Organisation has recommended semen from men with sperm antibodies as positive controls for the direct immunobead test [6], although it is silent about the MAR test, but unfortunately such men are not common. Samples used in external quality assurance (EQA) programmes [9] and commercially-available internal controls (eg SpermMar IgG Test Controls, FertiPro NV) are invariably serum because of the difficulty of collecting sufficient material with a high enough antibody titre. Serum samples must be analysed

using an indirect protocol [2], presenting difficulties with QC [11], and so such QC material in its native form is not suitable for use in the direct method. The present study has therefore developed a system using serum to provide the source of antibodies, combined with seminal plasma and donor semen to ensure the matrix closely resembled semen.

In making a pool of spiked seminal plasma, the first step was to determine a suitable amount of the antibody-positive serum to add to the seminal plasma, and this will need to be done with each batch of serum as the antibody titre may vary. If the antibody molecules are in too high a concentration, they will not form the cross-linked network required for incorporation of the sperm in to the agglutinated beads, even though the antibodies recognize the antigen. This phenomenon is called the prozone or hook effect and is well documented with a range of agglutination tests such as the Coombs test [12], and was clearly demonstrated in Figure 1 where the binding was actually reduced with increased volumes of serum beyond the optimum dose.

Motile sperm is required for the test to be valid, and so the use of a normozoospermic donor sample in the current protocol is crucial. The incubation of the semen for one hour at 37°C will significantly decrease motility [13], meaning that good motility is required in the first instance. There is also a 1:3 dilution of the test sample after the addition of beads and anti-serum test solutions, so that a good concentration of sperm is also required.

The inclusion of positive QC samples whenever a run is performed allows the laboratory to quantify the assay variability. The gathering of such quantitative information can also enable the laboratory to calculate measurement uncertainty for compliance to international standards such as ISO 15189 [14].

In conclusion, the use of spiked seminal plasma provides a simple means of QC for the MAR direct test. The samples would be suitable for both internal QC and EQA programmes, allowing the characteristics of the method to be determined and quantified.

#### Conflict of interest statement

We declare that we have no conflict of interest

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