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Application of a sperm survival test: Results from an external quality control programme

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ABSTRACT

Objective: The study aim is to determine which type of material – pipette tips or culture medium – is more appropriate for use in a cytotoxicity external quality control programme (CT-EQC).
Study design: The results of the participating laboratories in Spanish CT-EQC programme for human reproduction laboratories during the period 2013–2016 were analyzed. Per year, laboratories receiving three pipette tips and three aliquots of culture medium. All laboratories used the human sperm survival test to perform the bioassay. On average 48 laboratories took part in the programme each year. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall accuracy were calculated, with the corresponding 95% confidence intervals.
Results: Overall, for both products, sensitivity was higher than specificity, and NPV was higher than PPV. For laboratories participating for the first time in the CT-EQC, lower results were obtained in sensitivity and specificity in culture media than in pipette tips. However, in subsequent years, these differences disappeared. The PPV obtained for pipette tips was higher than that obtained for culture media (0.82 (0.77–0.87) vs 0.71 (0.66–0.76)). No relationship was recorded between the laboratories' accuracy in culture media and pipette tips (r = 0.026).
Conclusions: From a logistical standpoint, pipette tips are more appropriate than culture medium for use in a CT-EQC programme.

Introduction

It is very important that the materials used in assisted reproduction laboratories should not have any negative effect on the viability of gametes and embryos. In order to identify possible sources of reprotoxicity, guidelines recommend that the material be tested prior to use [1,2]. Various bioassays are available for this purpose [3–7], among which the human sperm survival test (hSST) is one of the most commonly used, due to its ease of use and ready availability [8,9].

The validity of the hSST is crucial to the quality achieved by an andrology and embryology laboratory, since the inadequate performance of this bioassay could lead to reprotoxic materials and/or media being used, leading to impaired results, or to the need to discard acceptable materials and/or media, thus increasing the cost of the process. In any case, a good bioassay system for materials and media would improve the results obtained and reduce costs.

To standardise bioassay systems, various scientific societies (American Association of Bioanalysts, Fertility Society of Australia, Association for the Study of the Biology of Reproduction) [10] encourage participation in a cytotoxicity external quality control programme (CT-EQC). In these programmes, participants receive laboratory materials, some of which have been contaminated with toxic substances, and must determine whether the material in

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question is toxic or not. Beforehand, the participating laboratories are totally unaware of whether the materials have been altered and of the quantity of materials affected.

These CT-EQC programmes have not yet been standardised, and so there are variations in incubation times [9], in the type of test used [11,12] and in the type of material sent for analysis [13]. The latter may include laboratory materials (pipette tips, transfer catheters, pasteur pipettes or petri dishes) and/or culture media. Differences have been observed in the results obtained [10], perhaps due, at least in part, to the wide variety of products used. To our knowledge, no previous studies have been conducted to determine whether the type of product used in a CT-EQC influences the results obtained.

The aim of this study is to compare two products (culture media and pipette tips) to determine which is more suitable for use in a CT-EQC programme, by analysing the results obtained over a four-year period in such a programme.

Material and method

The data used in this analysis were obtained during the period 2013–2016 from the Spanish EQC programme for human reproduction laboratories, organised by the Association for the Study of Reproductive Biology (ASEBIR). On average, 48 laboratories took part each year in the CT-EQC programme during this period. Pipette tips were evaluated by 37, 39, 54 and 64 laboratories, and embryonic culture media by 35, 40, 53 and 63 laboratories, in the years 2013, 2014, 2015 and 2016, respectively.

In all cases, participation was voluntary and independent of other reproduction laboratory EQC programmes organised or sponsored by ASEBIR (for embryo evaluation or semen analysis).

In this programme, which started in 2003, only laboratory materials were evaluated [10]. From 2013, each consignment for evaluation consisted of culture media and material (pipette tips).

The study data were compared with those obtained from this same programme during the years 2003–07 [10] and with those obtained in the CT-EQC programme conducted by the American Association of Bioanalysts from 2013 to 2016 [14].

The reagent used to add toxicity to the materials sent for CT-EQC analysis was chlorhexidine (Sigma-Aldrich, USA), a liquid disinfectant belonging to the bis(biguanide) family. On contact with biological material, it causes the precipitation of nucleic acids, protein denaturation and cell lysis. The culture medium was made toxic by the addition of 0.01% chlorhexidine, and the pipette tips were incubated with 2% chlorhexidine for 24 h.

The media were handled under sterile conditions and the pipette tips were sterilised in an autoclave prior to the toxic treatment. All materials had previously been tested by the programme organisers, who applied the hSST to verify the toxicity of the products sent, observing reductions in mobility >50% of the initial level at 24 h after contact between the material to be evaluated and the semen sample.

Each year during the programme, three samples of embryonic culture media, M2 medium with HEPES (Sigma-Aldrich, USA) and three pipette tips (Daslab, Spain), were sent to the participating laboratories. The results for one of the pipette tips are not presented in this paper because the treatment in this case was not consistent over the years analysed.

The materials were distributed to the participating laboratories by courier, as ordinary deliveries and using appropriately-labelled padded envelopes. The characteristics and specifications of the EQC programme were also included in the envelope. The pipette tips were sent at room temperature and the culture media were delivered under refrigerated conditions. The consignments were always sent on a Monday to avoid delivery delays. The laboratories were instructed to keep the media refrigerated until evaluation, which in any case should be performed as soon as possible.

The participating laboratories were totally unaware of how many of the products had been treated. All the laboratories used the hSST to assess the toxicity of the materials.

The results obtained by each laboratory were communicated via a website established for this purpose. The participating laboratories were asked to state whether, in their opinion, the products tested were toxic or non-toxic for use in an assisted human reproduction laboratory.

To analyse the responses made by the laboratories during the four years of the CT-EQC, the sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) and also the total agreement of the bioassays were calculated, together with the corresponding 95% confidence intervals.

Sensitivity is defined as the proportion of toxic materials detected by the bioassay; specificity is the proportion of non-toxic materials detected; VPP is the proportion of bioassays that correctly detect toxicity, and VPN is the proportion of bioassays

Table 1
Sensitivity, specificity, positive and negative predictive values and overall agreement between the CT-EQC programmes conducted by ASEBIR (2013–16), ASEBIR (2003–07) [10] and AAB [14].

	ASEBIR (2013–16)		ASEBIR (2003–07)	AAB
	Tips	Culture media	Materials[a]	Culture media
Sensitivity	0.93 (0.89–0.97)	0.87[b] (0.83–0.91)	0.83 (0.75–0.91)	0.97 (0.96–0.98)
Specificity	0.79 (0.73–0.85)	0.76[b] (0.71–0.81)	0.68 (0.59–0.77)	0.94 (0.93–0.98)
Negative predictive value	0.92 (0.88–0.96)	0.90[b] (0.87–0.93)	0.84 (0.76–0.92)	0.97 (0.96–0.98)
Positive predictive value	0.82[c] (0.77–0.87)	0.71[b] (0.66–0.76)	0.67 (0.59–0.77)	0.94 (0.93–0.98)
Overall agreement	0.86 (0.83–0.90)	0.81[b] (0.77–0.84)	0.75 (0.68–0.81)	0.96 (0.95–0.96)

(95% confidence interval).
[a] The following materials were evaluated: tips, gloves, dishes, catheters.
[b] $p \leq 0.05$ ASEBIR vs AAB.
[c] $p \leq 0.05$ ASEBIR (tips vs culture media).