Interlaboratory variability of the indirect mixed antiglobulin reaction in the assessment of antisperm antibodies

The first step in controlling the accuracy of a biological variable is establishment of a reliable, reproducible, standardized test. The most important procedure in the evaluation of immunologic male infertility is analysis of antisperm antibodies. These antibodies influence sperm functions by binding to the sperm membrane (1, 2).

Several studies have investigated the clinical relevance of different tests, but no test was found to represent a gold standard. Comparison of tests is hampered by the fact that antisperm antibody assays are insufficiently standardized in terms of specificity, sensitivity, and objectivity (3–5).

To overcome these difficulties, studies for external quality control for antisperm antibodies detection were done (6, 7). The tests were found to discriminate between samples that were positive for antisperm antibodies and those that were negative, but the interlaboratory variation of values was wide. As a consequence, claim for quality assessment to achieve accuracy, precision, competence, and sufficient for a correct application and interpretation in the diagnostic process for infertility is necessary (8).

One of the tests most commonly used for antisperm antibodies detection is the mixed antiglobulin reaction (9). A standardized procedure was recommended by the World Health Organization (10). The mixed antiglobulin reaction test can be performed directly and indirectly. In the direct method, antisperm antibodies that are fixed to the motile sperm of the patient are detected. The indirect test uses healthy donor spermatozoa as antigens to which soluble antisperm antibodies are attached before the test.

In a preliminary study, we evaluated the intraassay and interassay variation of the indirect mixed antiglobulin reaction test. We focused on antisperm antibodies in seminal plasma because antisperm antibodies in sera occur in a large proportion of infertile and fertile men and are not related to infertility.

Seminal plasma samples from 10 patients who were referred to the Department of Andrology of the University Hospital Marburg for infertility investigations were stored at −20°C. The samples were shipped in dry ice to the 10 participating laboratories and stored at −20°C until assayed. All participating laboratories used routinely the indirect mixed antiglobulin reaction test for antisperm antibodies.

Table 1 shows the results of the indirect mixed antiglobulin reaction test at the laboratories. Results of the test on the same samples varied greatly at different laboratories. For example, seminal plasma D showed results between 1% and 100%, whereas sample B displayed results between 6% and 100%. The use of different donor spermatozoa by each laboratory in the indirect mixed antiglobulin reaction test provides high interassay variation.

A high mean deviation (> 40%) was seen between the results obtained by different laboratories. According to multivariate statistical tests, highly significant main effects were observed among the 10 laboratories and seminal plasma samples (P < .005). Significant reciprocal effects between the laboratories and the seminal plasma samples (P < .005) were also noted.

“Quality assurance is a vital prerequisite of good laboratory practice. The application and continuous scrutiny of adequate internal quality control measures are essential to ensure that laboratory performance is consistent from day to day. External quality assessment is particularly important to verify the accuracy and reliability of laboratory results. Consequently, without appropriate IQC (internal quality control) and external quality control (EQC), laboratory results can have no true validity” (11).

The quality of methods in the andrology laboratory depends on several key issues. First, a reliable, reproducible, standardized test that is easy to perform should be available. Second, reference values for the variable should be provided. Finally, there should be a mechanism for measuring improvement of quality (12).

We investigated the variability of the results of the indirect mixed antiglobulin reaction test across laboratories. All participating laboratories examined identical seminal plasma samples containing antisperm...
antibodies by using a standardized indirect mixed antiglobulin reaction test. Large differences among the results were found. This variability stems from the methods of test system and the lack of standardization of sperm, rather than from methodologic differences among the participating laboratories or in test performance.

Each laboratory performed the test using different donor sperms. In one laboratory, different donors might be used for the test at different time points. The comparability of the results is therefore poor. This is a problem not only of structure and process quality but also of individual interactions between seminal antisperm antibodies and donor sperm antigen structure, which seem to influence the test results.

The experience with external quality control in andrology is limited, and large differences were found between laboratories when identical samples were assessed (13). For detection of antisperm antibodies, external quality control schemes have not yet been successfully applied. The management of antisperm antibodies requires further attention to ensure that patients are treated effectively and safely, because providing treatment on the basis of imprecise tests is of dubious benefit (14).

The identification of human sperm antigens is important for understanding the mechanism by which antisperm antibodies may impair the capacity of sperm for fertilization. Focus should be placed on expansion of the knowledge about immunologic sperm membrane proteins (15, 16). Development of methods to accurately detect how antisperm antibodies interact with sperm antigens that are relevant to fertilization will allow accurate treatment.

Appendix: Participating Laboratories

Prof. Dr. F. Comhaire, Andrology Unit Department of Endocrinology, Academich Ziekenhuis, Ghent, Belgium; Prof. Dr. F. Dondero, Department of Andrology, Policlinico “Umberto I” University of Rome, Rome, Italy; Prof. Dr. H.-J. Glander, Department of Andrology, University Hospital, Leipzig, Germany; Prof. Dr. G. Haidl, Department of Dermatology, University Hospital, Bonn, Germany; Dr. C. Bohring and Prof. Dr. W. Krause, Department of Andrology, University Hospital, Marburg, Germany; Prof. Dr. U. Kvist, Andrology Unit, Reproductive Medical Centre, Karolinska Hospital, Stockholm, Sweden; Dr. F.-M. Köhn and Prof. Dr. J. Ring, Department of Dermatology and Allergology, Technical University Munich, Munich, Germany; Prof. Dr. G. Schreiber, Department of Dermatology, University Hospital, Jena, Germany; Dr. C. Spiessens and Prof. G. Verhoeven, Leuven University Fertility Center, Leuven, Belgium; Dr. P. Huwe and Prof. Dr. W. Weidner, Department of Urology, University Hospital, Giessen, Germany.

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References


