# Molecular diagnosis of Y chromosome microdeletions in Europe: state-of-the-art and quality control

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**The polymerase chain reaction (PCR) screening of microdeletions of the Y chromosome has become an important diagnostic step in the work-up of male infertility. However, there is no agreement about how this diagnosis should be performed. There are suggestions that the large variation in deletion frequency reported in the literature could be due to the various selection criteria of the patients analysed, although methodological aspects may play a role as well. As for other genetic diseases, molecular diagnosis of Y chromosome microdeletions should be controlled by adopting strict internal quality control measures and by participating in external quality assessment schemes. Such an external quality assessment project is presently being organized jointly by the European Academy of Andrology and the European Molecular Genetics Quality Network. Three preliminary trials have given a state-of-the-art picture of the diagnostic performance in various European laboratories, showing an overall rate of misdiagnosis of ~5% for both *AZF*b and *AZF*c regions, and providing data useful in the generation of guidelines for the molecular diagnosis of Y chromosome microdeletions.**

*Key words:* assisted reproduction/azoospermia factor/male infertility/quality control/Y chromosome deletion

## Introduction

The molecular analysis of microdeletions of the Y chromosome is currently performed in many diagnostic and research laboratories as part of work-up of male infertility. Following the discovery of the *DAZ* gene (Reijo *et al*., 1995) and the description of the three *AZF* regions (Vogt *et al*., 1996), a large number of studies have shown that *AZF* deletions are responsible for male infertility characterized by azoospermia or severe oligozoospermia and that such deletions can be transmitted to the offspring via intracytoplasmic sperm injection (ICSI) and testicular sperm extraction (TESE) (for reviews,

**Table I.** List of participants

| Institution | Country |
|---|---|
| Clinical Genetics, St. Johannisspital, Salzburg | Austria |
| Center for Medical Genetics, University Hospital, Gent | Belgium |
| Center for Medical Genetics, University Hospital, Brussels | Belgium |
| Center for Human Genetics Campus Gasthuisberg, Leuven | Belgium |
| The Family Federation of Finland, Helsinki | Finland |
| Institute of Human Genetics of the University, Leipzig | Germany |
| Institute of Human Genetics of the University, Göttingen | Germany |
| Institute of Human Genetics and Anthropology of the University, Düsseldorf | Germany |
| Institute of Reproductive Medicine of the University, Münster | Germany |
| Institute of Human Genetics of the University, Magdeburg | Germany |
| Praxis Prof. Held, Hamburg | Germany |
| Endokrinologische Praxisgemeinschaft, Hamburg | Germany |
| Gemeinschaftpraxis Dr Waldemeier, München | Germany |
| Institute of Human Anatomy, Tor Vergata University, Roma | Italy |
| Department of Andrology, University of Catania | Italy |
| Institute of Biology and Genetics of the University, Chieti | Italy |
| Institute of Medical Pathology of the University, Padova | Italy |
| Andrology Unit, University of Firenze | Italy |
| Institute of Histology, Embryology and Genetics of the University, Modena | Italy |
| S. Chiara Hospital, Pisa | Italy |
| Andrology Unit, Karolinska Hospital, Stockholm | Sweden |
| Center of DNA Diagnostics, University Hospital, Utrecht | The Netherlands |
| Department of Clinical Genetics, Erasmus University, Rotterdam | The Netherlands |
| Institute of Human Genetics, Amsterdam | The Netherlands |
| Institute of Human Genetics, Leiden | The Netherlands |
| Department of Medical Genetics of the University, Groningen | The Netherlands |
| Department of DNA Diagnostics, Maastricht | The Netherlands |
| Department of Human Genetics, University Hospital, Nijmegen | The Netherlands |
| Regional Cytogenetics Centre, Southmead Hospital, Bristol | UK |

see Kostiner *et al*., 1998; Simoni *et al*., 1998; Vogt, 1998; Kausz and McElreavey, 1999; Ma *et al*., 2000; Maurer and Simoni, 2000).

With the aim of providing an additional diagnostic tool for male infertility, many laboratories have started offering polymerase chain reaction (PCR)-based deletion analysis using in-house methods. This occurred in the absence of consensus on the procedure and on which loci on the Y chromosome should be analysed, as reflected by the lack of standardized diagnostic kits on the market. Based on the results of quality control trials involving other diagnostic procedures in molecular genetics, it is reasonable to expect that a portion of the diagnoses concerning the Y chromosome microdeletions is wrong, leading to false positive (when non-existent deletions are diagnosed) or false negative (when deletion are not detected) results (Losekoot *et al*., 1999; Dequeker and Cassiman, 2000).

In 1997, on the initiative of the European Academy of Andrology (EAA) training centre of Münster, a pilot trial was started in which DNA samples were exchanged between laboratories in several European countries (Simoni, 1998). The aims of the trial were: (i) to gain information about the molecular diagnosis of Y-chromosomal microdeletions; (ii) to cross-check the reliability of the various diagnostic protocols; and (iii) to collect data useful for a minimal standardization of this procedure.

Three trials have been completed in 2 years. Two DNA samples were sent to the participating laboratories, which were

**Table II.** DNA samples distributed in the three trials

| Trial 1 | sample 1a: *AZF*c deletion |
|---|---|
| | sample 1b: proven father |
| Trial 2 | sample 2a: *AZF*c deletion |
| | sample 2b: idiopathic azoospermia |
| Trial 3 | sample 3a: *AZF*b deletion |
| | sample 3b: *AZF*c deletion |

simply requested to perform the diagnosis according to their current protocol. The results of these trials, presented here, formed the basis for the generation of guidelines for the molecular diagnosis of Y chromosome microdeletions (Simoni *et al*., 1999).

## Diagnosis of Y chromosome microdeletions: state of the art

In all, 20 laboratories participated in the first trial, 25 in second trial and 29 in the third trial (Table I). Six DNA samples were used in this study. DNA was extracted from EDTA blood leukocytes from patients attending the infertility clinic of the Institute of Reproductive Medicine in Münster ($n = 5$) using the Nucleon BACC1 kit (Amersham, Braunschweig, Germany) and stored in aliquots of ~100–200 μg/ml. One sample was provided by a participating laboratory. The patients gave their informed, written consent to donate a blood sample for this study. The characteristics of the six DNA samples are summarized in Table II. Two DNA samples were sent by mail

**Table III.** Number of laboratories analysing the individual loci

| Name (sY) | Locus | Trial 1 | Trial 2 | Trial 3 |
|---|---|---|---|---|
| | ZFX/ZFY | 1 | 1 | 1 |
| 14 | SRY | 2 | 4 | 8 |
| 16 | DYS242 | 4 | 4 | 5 |
| 17 | | 0 | 0 | 1 |
| 18 | | 0 | 0 | 1 |
| 70 | | 0 | 0 | |
| 55 | DYF67SI | 1 | 0 | 0 |
| 72 | DYS266 | 4 | 4 | 5 |
| 75 | DYF27 | 1 | 0 | 0 |
| 78 | DYZ3 | 1 | 2 | 5 |
| 81 | DYS271 | 2 | 2 | 4 |
| 82 | DYS272 | 1 | 1 | 2 |
| 86 | DYS148 | 10 | 12 | 13 |
| 85 | DYS274 | 1 | 1 | 1 |
| Y6HP35PR | DYS274 | 1 | 1 | 0 |
| 84 | DYS273 | 16 | 21 | 24 |
| DFFRY | | 0 | 0 | 1 |
| 87 | DYS275 | 3 | 3 | 4 |
| 88 | DYS276 | 1 | 1 | 2 |
| 182 | KAL-Y | 2 | 2 | 4 |
| 151 | KAL-Y | 1 | 2 | 3 |
| 94 | DYS279 | 2 | 3 | 4 |
| 95 | DYS280 | 2 | 5 | 6 |
| 97 | DYS281 | 1 | 1 | 3 |
| 98 | | 0 | 0 | 1 |
| 100 | | 0 | 1 | 3 |
| 102 | DYS198 | 2 | 3 | 4 |
| 105 | DYS201 | 1 | 1 | 3 |
| 109 | DYF43SI | 1 | 1 | 3 |
| 112 | DYF46SI | 1 | 1 | 1 |
| Y6D14PR | DY5205 | 1 | 1 | 3 |
| Y6BAH34PR | DYS206 | 1 | 1 | 3 |
| 116 | | 0 | 0 | 1 |
| 117 | DYS209 | 5 | 6 | 9 |
| Y6PHC54PR | | 1 | 1 | 2 |
| 124 | DYS215 | 1 | 1 | 1 |
| 125 | DYS216 | 1 | 2 | 2 |
| 127 | DYS218 | 9 | 11 | 15 |
| 128 | DYS219 | 1 | 1 | 2 |
| 131 | DYS222 | 1 | 2 | 2 |
| 130 | DYS221 | 2 | 3 | 4 |
| 129 | DYS220 | 1 | 1 | 2 |
| 132 | DYS7 | 3 | 2 | 3 |
| 134 | DYS224 | 10 | 12 | 18 |
| 164 | DYF65SI | 2 | 2 | 2 |
| 138 | DYF49SI | 1 | 2 | 0 |
| 139 | DYF49SI | 1 | 2 | 0 |
| 139 | DYS227 | 0 | 1 | 1 |
| 141 | DYS229 | 1 | 1 | 1 |
| 142 | DYS230 | 2 | 2 | 2 |
| 143 | DYS231 | 5 | 9 | 14 |
| | MK5 | 1 | 1 | 3 |
| | RBM1 | 0 | 1 | 2 |
| 145 | | 0 | 0 | 1 |
| 155 | DAZ | 2 | 4 | 2 |
| 150 | DYS235 | 2 | 2 | 1 |
| 153 | DYS237 | 3 | 5 | 11 |
| 152 | DAZ | 4 | 6 | 7 |
| 220 | | 1 | 1 | 1 |
| 232 | DAZ | 1 | 1 | 1 |
| 233 | DAZ | 1 | 1 | 1 |
| Fr15-lipr | | 1 | 1 | 2 |
| Y6HP52PR | DYS239 | 1 | 1 | 3 |
| 147 | DAZ | 6 | 8 | 13 |
| 240 | | 2 | 1 | 1 |
| 242 | DAZ | 1 | 1 | 2 |
| 148 | DAZ | 1 | 2 | 2 |
| 146 | | 0 | 0 | 2 |
| 156 | DYS239 | 1 | 2 | 1 |

Table III Continued.

**Table III.** Continued.

| Name (sY) | Locus | Trial 1 | Trial 2 | Trial 3 |
|---|---|---|---|---|
| 231 | | 1 | 0 | 0 |
| 149 | DAZ | 4 | 6 | 6 |
| 206 | | 1 | 0 | 0 |
| 208 | DAZ | 1 | 2 | 2 |
| 254 | DAZ | 15 | 19 | 23 |
| 255 | DAZ | 12 | 16 | 18 |
| 277 | DAZ | 5 | 8 | 5 |
| 279 | DAZ | 1 | 1 | 0 |
| 283 | DAZ | 3 | 4 | 3 |
| | SPGY(DAZ) | 2 | 3 | 3 |
| 243 | | 3 | 2 | 4 |
| 236 | | 3 | 2 | 1 |
| 272 | | 2 | 0 | 0 |
| 273 | | 1 | 2 | 0 |
| 269 | | 0 | 1 | 2 |
| 202 | | 4 | 2 | 3 |
| 247 | | 2 | 1 | 0 |
| CDYa | | 0 | 0 | 1 |
| 157 | DYS240 | 4 | 4 | 8 |
| 167 | DYS248 | 1 | 0 | 0 |
| 158 | DYS241 | 4 | 8 | 10 |
| 166 | DYS247 | 2 | 1 | 1 |
| 160 | DYZ1 | 3 | 2 | 5 |

to the individual laboratories in each trial. All samples were received and PCR analysis was possible in each case.

Each laboratory was requested to perform PCR diagnosis of the Y chromosome according to its usual protocol. The individual protocols were reported to the centre only in part. Seven laboratories used a multiplex PCR approach, five simplex PCR, while the format adopted by the remaining 17 laboratories was not specified.

Although reporting is a very important component of the diagnostic procedure which is considered in quality assessment schemes of genetic diseases, the participating laboratories were not requested to return official reports in English at this stage. Results were returned to the centre by fax and/or mail ($n = 21$) or by e-mail ($n = 8$). Official reports written in English were returned by nine centres, all laboratories belonging to human genetics institutions already involved in other quality control programmes for genetic diseases.

At the beginning of the study, no specific indications were given concerning internal quality control. After termination of the second trial it was suggested that the laboratories should adopt minimal quality control rules, i.e. to use a blank, a female DNA sample and a DNA sample from a male with proven fertility in each PCR reaction. Moreover, the laboratories were advised to amplify a locus on the short arm of the Y chromosome. At the end of the third trial, 18 of 29 laboratories (62%) used a marker specific for the short arm of the Y chromosome. Information about compliance with the other indications was available only from some laboratories: 13 laboratories regularly ran a blank, 12 laboratories ran a female DNA sample and 12 laboratories ran a fertile male DNA sample. The remaining laboratories did not provide similar information, thus making accurate assessment of internal quality control impossible. From the rate of misdiagnosis (see below) it was clear that internal quality control was
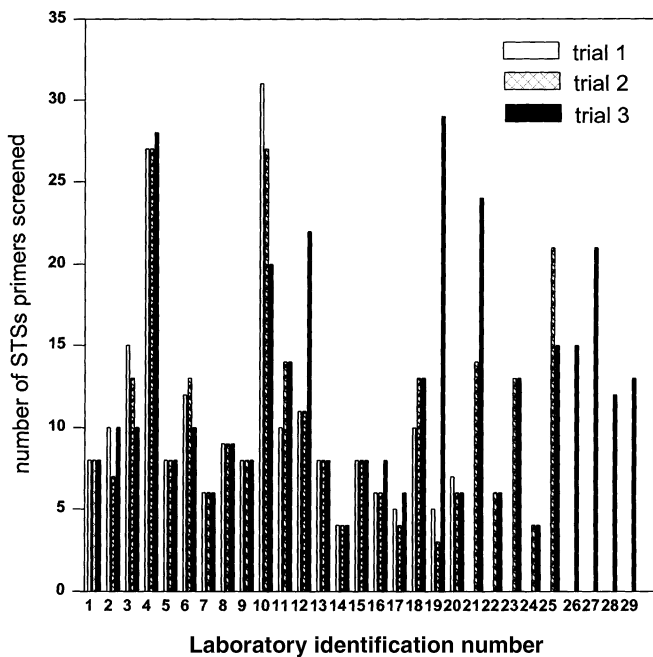
**Figure 1.** Number of sequence-tagged site (STS) primers used in *AZF* diagnostics by the laboratories participating in the external quality control assessment scheme.

insufficient in some laboratories. However, a spontaneous reduction of the rate of wrong results was observed from trial to trial, suggesting that the laboratories improved their internal quality control as a result of their participation.

The sequence-tagged site (STS) primers used by the participants in each trial are listed in Table III. No reason for the choice of primers was indicated. Overall, 90 different primers were employed in trial 1 (nine of which amplified STSs in the *AZF*a region, 23 in *AZF*b, 36 in *AZF*c and 22 other STSs), 77 primers in trial 2 (eight in *AZF*a, 24 in *AZF*b, 30 in *AZF*c and 15 in other regions) and 80 primers in trial 3 (nine in *AZF*a, 24 in *AZF*b, 36 in *AZF*c and 11 in other regions). Figure 1 shows the number of primers used by the individual laboratories in each trial. The number of STS primers varied from 3 to 29 (median = 8.5) and varied within 14 individual laboratories between the trials. The reason for changing the primer choice derived in part from the results of the first and the second trial, which showed inter-laboratory inconsistencies. A large number of STS primers reported in Table III are known to amplify sequences belonging to the *DAZ* gene cluster. These are: sY152, sY232, sY233, sY155, sY147, sY242, sY148, sY149, sY208, sY254, sY255, sY277, sY279, sY283, SGPY (Kostiner *et al*., 1998). The number of PCR markers amplifying *DAZ* varied between the laboratories from 1 to 9 (not shown).

The results returned by the participants were summarized in tables and distributed to the participants. Figure 2 show the results obtained by the individual laboratories from the analysis of sample 1b, and Figure 3 shows the results of sample 3a. The heterogeneity of the primer sets used is evident. Remarkably, none of the primers was common to all participants.

In the first trial all participants correctly diagnosed the deletion in *AZF*c of sample 1a, while two laboratories diagnosed a deletion incorrectly (i.e. obtained a false positive result) in

sample 1b (Figure 2). One of these two laboratories (no. 10) was unable to amplify as many as seven STSs in sample 1b, a result clearly at odds with the data obtained by the other participants. The second laboratory had a false positive result in only one STS. These data correspond to a rate of misdiagnosis for trial 1 of 5% (2/40 diagnoses). In trial 2, all laboratories diagnosed a deletion in *AZF*c correctly. However, one laboratory incorrectly diagnosed an additional microdeletion involving only one and the same STS in *AZF*a in both DNA samples (not shown) (rate of misdiagnosis: 2/50, 4%). In trial 3, all laboratories correctly diagnosed the deletion in *AZF*c in sample 3b, while three laboratories completely failed to diagnose the deletion in *AZF*b in sample 3a (Figure 3). This corresponds to a rate of misdiagnosis of 5.2% (three out of 58 diagnoses). Such false negative results were obtained using two STS primers by two laboratories (nos. 7 and 18 in Figure 3) and only one STS by one laboratory (no. 6).

Laboratory 4 produced 'uncertain' results for two STSs in trial 1 and for four STS in trial 3. Laboratory 15 returned 'uncertain' results for one STS and laboratory 26 for two STSs in trial two. The overall ratio of incorrect and dubious results is summarized in Figure 4. The percentage of wrong and dubious PCR results decreased progressively, partly because the use of some primers which gave inconsistent inter-laboratory results was abandoned. The use of STS primers sY55, sY75, sY231, sY272 and sY167 was spontaneously abandoned by the laboratories using them after the first trial (Table III). Among these primers, only sY272 had given inconsistent inter-laboratory results in trial 1. Further inter-laboratory discrepancies in trial 1 were found for the following primers: sY152, sY153, sY236, sY166 and sY147. After trial 2, the use of the following primers was abandoned: Y6HP35PR, sY150, sY138, sY279, sY273 and sY247 (Table III). Among them, sY150 and sY273 had shown poor inter-laboratory consistency.

## Necessity for standardization and quality control

The first obvious issue resulting from this survey is the extreme heterogeneity of the panels of PCR markers (number and type) used by the various laboratories. This reflects perfectly the heterogeneity of the protocol adopted in the published scientific papers. The criteria for choosing a given protocol are unknown and, in the absence of guidelines, each laboratory arbitrarily chooses and sets up its own method. The heterogeneity of the methods has been useful to identify unreliable STS markers, the use of which has been spontaneously abandoned by many laboratories, but renders the interpretation of the performance of the individual laboratories impossible.

Secondly, the choice of primers is unbalanced towards the *AZF*c region and, in particular, the *DAZ* gene. At least 15 *AZF* candidate genes have been described so far (Lahn and Page, 1997; Kostiner *et al*., 1998; Vogt, 1998) but their role in spermatogenesis is basically unknown. The large majority of microdeletions in infertile patients occurs in the *AZF*c regions, where the *DAZ* gene cluster is located. Moreover, *DAZ* was the first discrete gene described in this region for which a causal role in infertility was proposed (Rejio *et al*., 1995). These are probably the two main reasons for the preference
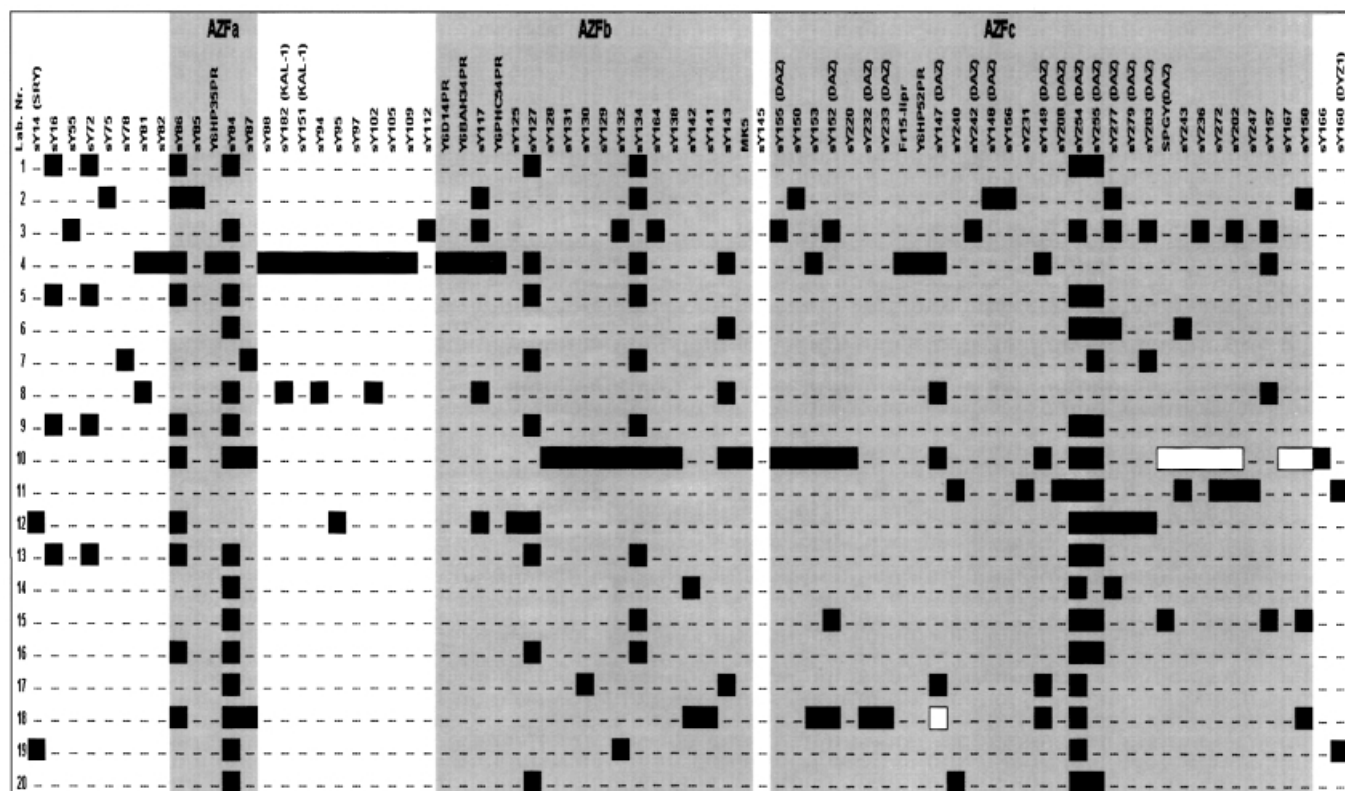
**Figure 2.** Results of the first trial, sample 1b (proven father). Black box = locus analysed and present; white box = locus analysed and absent; − = not analysed.

of PCR markers in this area for diagnostic purposes. Although, in principle, the choice of STS primers amplifying discrete genes would be preferable, the amplification of anonymous STS loci in diagnostic protocols is fully acceptable, provided that they cover clinically relevant regions of the Y chromosome and give reproducible results. Nevertheless, some form of standardization should be implemented, so that inter-laboratory variability can be better controlled.

The overall diagnostic accuracy, expressed in terms of sensitivity and specificity as calculated from the results of the three trials, is shown in Table IV. Although the methods employed are non-homogeneous, the overall sensitivity in detecting deletions in *AZF*b and *AZF*c is 97%, while, due to false positive results, the specificity is lower (91%). It is interesting to notice that, although no samples with *AZF*a deletion were distributed in these three trials, one laboratory misdiagnosed two deletions in *AZF*a (false positive) in the second trial.

The present data show that the diagnosis of samples with *AZF*c deletions has a good sensitivity since no false negative results were reported. On the other hand, microdeletions of the *AZF*c region can be occasionally diagnosed in normal samples, as suggested by the two cases where false positive results were reported (Table III). Obviously, the diagnostic specificity of *AZF*c deletions is independent of the number of primers used. In fact, one of the two false positive diagnoses of deletion in *AZF*c was generated from a large number of primers. In principle, the use of many primers in the same region prevents neither false negative results nor overestimation

of the rate of deletions. It has been reported that when PCR results showing deletions were checked by Southern blotting, the percentage of microdeletions in a group of patients fell from 13.2 to 3.2% (Ma *et al.*, 2000). Again, this stresses the necessity for rigorous internal quality control. Extrapolating these results to the clinical arena, it can be concluded that patients with deletions involving the *DAZ* gene are not expected to escape diagnosis, but deletions of the *AZF*c area can be incorrectly diagnosed in ~3–5% of men with a normal Y chromosome. This is particularly important in the case of azoo/oligozoospermic candidates for TESE/ICSI, where the genetic counselling and the decision whether to undergo assisted reproduction may depend upon the outcome of the genetic test (Annemiek *et al.*, 1999).

The diagnosis of samples with *AZF*b deletions is even more problematic. In fact, as many as three laboratories failed to make the correct diagnosis. Two laboratories failed by using the three most commonly employed primers, which were not found to present analytical problems to other laboratories. These results suggest poor diagnostic performance of the PCR protocol adopted by those laboratories for *AZF*b deletions and stress the need for a more stringent control of assay conditions by using appropriate control samples. This underlines the necessity of running negative controls (female DNA, blank) and a positive control (normal male DNA) in each assay in order to detect contamination or amplification of spurious bands promptly (Neumaier *et al.*, 1998). Internal controls are particularly important in avoiding false negative results, because if a band of the expected size is found to be present, the PCR reaction is usually
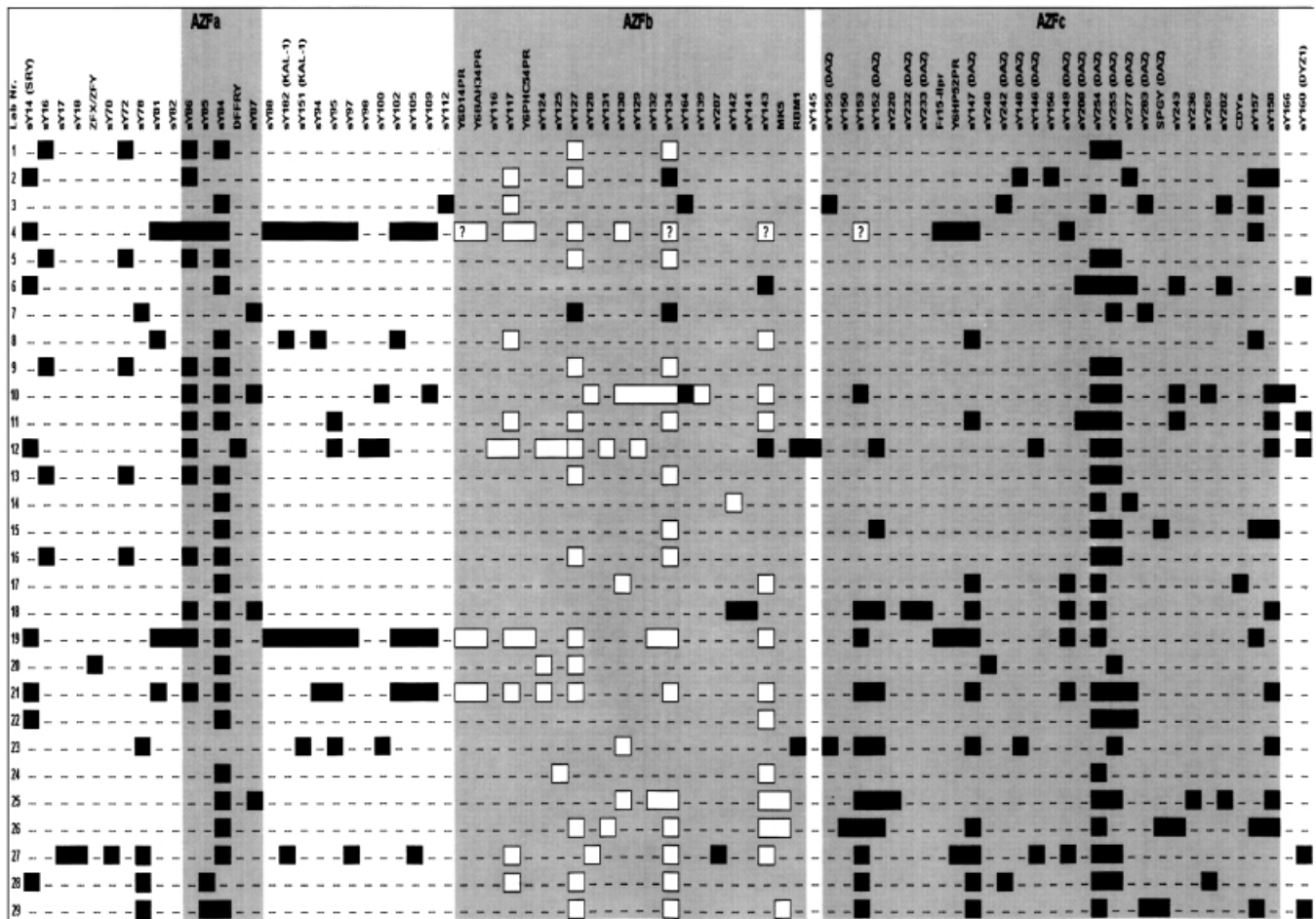
**Figure 3.** Results of the third trial, sample 3a (patient with *AZF*b deletion). Black box = locus analysed and present; white box = locus analysed and absent; – = not analysed.
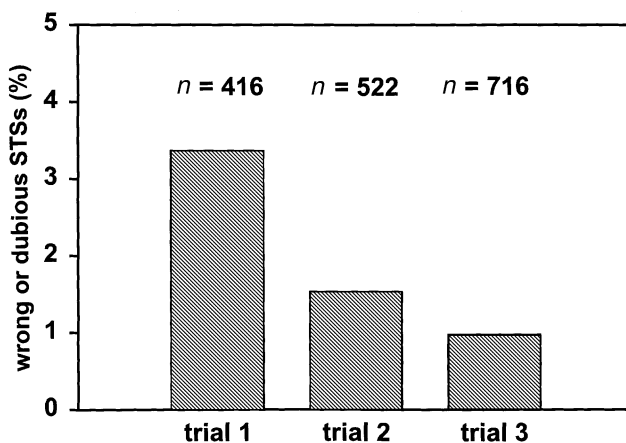


**Figure 4.** Error rate = percentage of wrong or dubious polymerase chain reaction (PCR) results in the three trials. The total number of sequence-tagged site (STS) primers used in each trial is given over the bars.

not repeated a second time, while samples showing deletions are usually reanalysed. Extrapolating these results to routine clinical use, one might expect a clinical underestimation of deletions in the *AZF*b regions of ~5%. Unfortunately, no sample with *AZF*a deletion was available at the time when these trials were organized, so that the diagnostic accuracy of *AZF*a deletions cannot be calculated.

The overall rate of misdiagnosis of *AZF*b and *AZF*c was 5%, a value within the range of misdiagnoses for other genetic tests (Dequeker and Cassiman, 1998; Losekoot *et al*., 1999). However, this value is probably an underestimate because other important aspects contributing to the accuracy of the diagnosis, e.g. sample drawing and storage, DNA extraction and, especially, clerical errors in reporting the results, have not been considered so far. As for the analytical procedure, in addition to unsatisfactory internal quality control, the use of STSs with poor inter-laboratory consistency could play a role in misdiagnosis. Moreover, some STS loci analysed by the participants are known to be polymorphic, i.e. are also found to be deleted in the normal population (Pryor *et al*., 1997; Vogt, 1998; Kent-First *et al*., 1999), and some primers amplify repetitive sequences on the Y chromosome. Far from contributing to an accurate and meaningful diagnosis, the use of such STS primers should be abandoned. It is expected that the adoption of strict internal quality control measures (Neumaier *et al*., 1998) and participation in an external quality control assessment scheme will reduce and should (eventually) eliminate misdiagnoses.

**Table IV.** Current diagnostic accuracy of *AZF* deletions

| Trial no. | Diagnoses (*n*) | False positives (FP) (*n*) | False negatives (FN) (*n*) | True positives (TP) (*n*) | True negatives (TN) (*n*) |
|---|---|---|---|---|---|
| 1 | 40 | 2 | 0 | 20 | 18 |
| 2 | 50 | 2 | 0 | 25 | 24 |
| 3 | 58 | 0 | 3 | 55 | 0 |
| Total | 148 | 4 | 3 | 100 | 42 |

Sensitivity:   TP / (TP + FN) = 97%
Specificity:   TN / (FP + TN) = 91%

### The EAA/EMQN laboratory guidelines for molecular diagnosis of Y-chromosomal microdeletions

After the completion of this study the participating laboratories were invited to evaluate the results and to contribute to the generation of laboratory guidelines for the molecular diagnosis of Y chromosome microdeletions. Such guidelines (Simoni *et al.*, 1999) have been compiled with the valuable contribution of both andrologists and geneticists of several European countries and represent a first step towards the standardization and improvement of the quality of this procedure. The guidelines have been designed keeping in mind that a diagnostic test should be accurate, easy to perform, reproducible and, possibly, inexpensive. It is not to be expected that the proposed protocol will detect *all* deletions, but no diagnostic test does that in molecular genetics. For instance, the current molecular diagnosis of cystic fibrosis detects 'only' 80% of mutations. These guidelines are now officially recognized and promoted by both the EAA and the European Molecular Genetics Quality Network (EMQN) and can be downloaded free from the respective home pages (http://www.blackwell-science.com/uk/society/eaa/ and http://www.emqn.org/). The guidelines are by no means an immutable precept. On the contrary, the rapid expansion of the information in this field demands frequent revisions, in the light of the upcoming knowledge of the complete sequence of the Y chromosome and of the function of its genes. For instance, the recent description of the genomic organization of the *DAZ* gene cluster revealed four individual genes arranged in two clusters each comprising an inverted pair (Saxena *et al.*, 2000). The four genes differ in their length. In the near future it will be possible to design amplification strategies detecting the individual genes to test the hypothesis that some cases of infertility are associated with partial *DAZ* gene deletions. Obviously, future diagnostic protocols will have to consider these developments and include the amplification of discrete genes whenever it is clinically relevant. For the time being the EAA and the EMQN are jointly organizing an external quality control assessment scheme, based on the distribution of well-characterized DNA samples of good quality and on the evaluation of the results by a panel of expert assessors according to the rules followed by the EMQN for the external quality control schemes of other genetic diseases. It should be pointed out that the persons involved in this effort are doing this on a voluntary basis and have no personal interest in its promotion other than that of providing a service to the scientific community and to the diagnostic laboratories.

### Perspectives and conclusions

While the option for an infertile couple of whether to undergo ICSI or TESE/ICSI can remain uninfluenced by the diagnosis of a microdeletion of the Y chromosome (Annamiek *et al.*, 1999; Meschede *et al*, 2000), Y deletion analysis is assuming an important prognostic value about the probability of recovering sperm upon TESE in azoospermic men (Krausz *et al.*, 2000). It can be easily foreseen that this diagnostic procedure will be continued in the future. In the meantime the first diagnostic kits have appeared on the market and many laboratories might choose to adopt such commercial methods. Similar to the in-house methods, commercial kits should be carefully validated before routine clinical use. Moreover, each diagnostic laboratory should participate in an external quality control scheme. For many other genetic diseases, in many countries, diagnostic laboratories have to undergo a certification procedure or at least demonstrate that they participate in a programme for proficiency testing. Soon this will probably also be true for the molecular diagnosis of the Y chromosome. The experience in external quality control of other genetic diseases shows that the error rate improves during quality control trials but only less than half of the laboratories make no mistakes (Dequeker and Cassiman, 2000). The adoption of well-validated, reliable and reproducible diagnostic protocols and the continuous, qualitative improvement deriving from taking part in external assessment schemes are necessary for diagnosis of the Y chromosome as well.

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