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TECHNICAL NOTE

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Probability of Exclusion in Paternity Testing: Time to Reassess*

ABSTRACT: The average exclusion probability is a measure of efficiency in paternity testing; it refers to the *a priori* ability of a battery of tests to detect paternity inconsistencies. This parameter measures the capacity of the system to detect a false accusation of paternity. Traditionally, this average exclusion probability has been estimated as the probability of excluding a man who is not the father *by an inconsistency* in at least one of the studied loci. We suggest that this criterion should be corrected, as currently the presumed father is excluded when at least three genetic inconsistencies are found with the child being tested, not just one. This change of criterion has occurred because of the use of microsatellite loci, whose mutation rates are much greater than those of the coding genes used previously in paternity studies. We propose the use of the average probability of exclusion for at least three loci (not only one), as an honest measure of the combined probability of exclusion of several loci, and we propose an algebraic expression to calculate it.

KEYWORDS: forensic science, forensic genetics, paternity testing, exclusion probability, minisatellites, STRs, paternity exclusion

It is currently possible to attribute or exclude biological paternity with security, thanks to the analysis of the hypervariable regions of the genome. The most informative are the variable number of tandem repeat (VNTR) (minisatellites) and single tandem repeats (STR) (microsatellites), which have a large number of alleles and high levels of heterozygosity (1). However, these loci have high levels of mutation (from 0.0005 to 0.007 per generation) (2) compared with the coding genes (blood groups, HLA antigens, polymorphic proteins) that were previously utilized in parentage and forensic studies. When these expressed markers were used in paternity studies, the requirement to exclude an alleged father was that he had to be genetically inconsistent with the child at one or more of the analyzed genes. Today, however, taking into consideration the high mutation rates of mini- and microsatellites, no one would exclude an alleged father because he has only one genetic inconsistency with the child. Nowadays, for the exclusion of an alleged father he should not have the paternal obligate alleles found in the offspring in three or more independent locus tests (3). The American Association of Blood Banks Report Summary for Testing in 2003 reports 67 cases with double mutations as paternity inclusions among the 352,632 paternity cases studied (0.019%); they observed similar figures in 2002 and 2001 (4).

The criterion to evaluate the efficiency of the genetic markers used in paternity studies is their *a priori* average exclusion probability (5,6). This is the probability that the chosen system is capable of excluding a man falsely indicated as the father. Traditionally, the joint *a priori* average exclusion probability (PEC) provided by a group of independent genetic markers was estimated as the probability of excluding a man who is not the father for at least one locus, using the formula (7):

$$\mathrm{PEC} = 1 - \prod_{i=1}^{n} (1 - \mathrm{PE}i)$$

where PE*i* is the specific exclusion probability of the *i*th genetic marker and $\Pi(1 - \mathrm{PE}i)$ means $(1 - \mathrm{PE}_1) \times (1 - \mathrm{PE}_2) \times (1 - \mathrm{PE}_3) \times \cdots \times (1 - \mathrm{PE}_n)$ from the locus $i = 1$ to the *n*th locus.

The above formula is no longer the correct way to calculate PEC, as now the requirement includes genetic inconsistencies for at least three independent loci. This joint average exclusion probability (PEC3) may be calculated by the following expression:

$$\mathrm{PEC3} = 1 - [\mathrm{PE}(0) + \mathrm{PE}(1) + \mathrm{PE}(2)]$$

where PE(0), PE(1), and PE(2) are the probabilities of excluding the presumed father for exactly 0, 1, or 2 loci respectively, when he is not the biological father.

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The value of PE(0) is obtained by the above expression (PEC), while the other components may be estimated by

$$\mathrm{PE}(1) = \sum_{i=1}^{m} \mathrm{PE}_i \prod_{\substack{j=1 \\ j \neq i}}^{m} (1 - \mathrm{PE}_j)$$

$$\mathrm{PE}(2) = \sum_{i=1}^{m} \sum_{j<i}^{m} \mathrm{PE}_i \mathrm{PE}_j \prod_{\substack{k=1 \\ k \neq i \\ k \neq j}}^{m} (1 - \mathrm{PE}_k)$$

Here PE_i, PE_j, and PE_k are the exclusion probabilities of the *i*th, *j*th, and *k*th loci, respectively and ΣPE_i refers to $(PE_1)+(PE_2)+(PE_3)+\cdots+(PE_m)$ where PE_i to PE_m are the specific probability of exclusion of each of the m independent loci analyzed.

The probability of excluding a falsely accused father for at least three independent loci (PEC3) is less than the probability of excluding him for one locus, and the difference is not trivial. For example, using the allele frequencies estimated in the Chilean population (8–10), an exclusion probability of 99.9% for at least one locus is obtained by analyzing 9 or 10 independent STRs, while it requires 14 or 15 STRs to obtain a 99.9% exclusion probability for at least three loci. We propose the use of PEC3, the average probability of exclusion for at least three loci, as an honest measure of the *a priori* ability of a battery of tests to detect paternity inconsistencies that is to detect a false accusation of paternity.

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