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DEVELOPMENT, PRESENT STATE AND PROSPECTS OF DNA ANALYSIS IN THE STATE CRIMINAL INVESTIGATION DEPARTMENT OF MECKLENBURG-WESTERN POMERANIA

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Summary

Following a judgement of the Federal High Court of Justice on the admissibility of DNA analysis in criminal proceedings, the division of Forensic Biology of the Forensic Institute of the State Criminal Investigation Department of Mecklenburg – Western Pomerania began carrying out DNA analysis of traces as part of investigation routines in August 1996. Prior to that, this methodology had been built up and validated for about a year. At the beginning, only two autosomal DNA systems were analysed, a further six were added until May 1997. At that point in time, the division employed two technical assistants and one expert. In 1997 575 traces and materials for comparison were collected and a total of 2,277 amplifications were carried out.

In March 1997 the Criminal Proceedings Law Amendment Act regarding DNA analysis established a clear-cut legal base for molecular genetic investigations in Federal Germany: from now on a judge will have to order DNA analysis investigations of traces and personal material in criminal proceedings.

In March 1998 a nationwide DNA analysis database (DAD) was established at the Federal Office of Criminal Investigation (BKA). This requiered a change in the methods of analysis. To meet the criteria for input into and searches within the database, two ABI PRISM® Genetic Analysers 310 were purchased from Applied Biosystems. This increased the number of autosomal DNA systems analysed in our laboratory to twelve, including the five core systems of the nationwide DNA analysis database. Since August 1998 the state of Mecklenburg – Western Pomerania has contributed inputs for and searched traces and personal data files in the DAD. Some 285,000 data sets have so far been included in the nationwide DAD, of which about 4,000 from our own laboratory.

Today we are in a position to carry out DNA analyses of the following trace materials: blood, saliva, sperm, vaginal epithelial cells, skin epithelial cells, urine, tissue and faeces. Four ABI PRISM® Genetic Analysers 310 from Applied Biosystems are available for these investigations. The division now employs two experts, four assistants and one administrative assistant. Routine investigations cover seventeen autosomal DNA systems, six YSTRs as well as amelogenine. In 2002 some 4,400 traces and materials for comparison were collected. Also, nearly 11,000 amplifications were carried out.

For the future it is intended to add morphological and DNA analytical investigations of hairs to our analytical spectrum. The examination of trace materials with a low content of DNA or with partly degenerated DNA shall be improved by introducing real time PCR. There are also plans to extend investigations of Y-chromosomal systems in cases of rape and of sexual assaults.

Technical necessities for such investigations shall be provided in a new building for our institute in 2006.

Introduction

All DNA analyses carried out by the State Criminal Investigation Department of Mecklenburg – Western Pomeranian fall under the responsibility of the serology sub-section. Together with the sub-sections of textile analysis and general biology they make up the biology section, one of four sections comprising the Forensic Institute of the State Criminal Investigation Department. The serology sub-section represents the central analytical laboratory of the state police authority for biological traces, secured in the context of criminal offences.

First, and by way of an introduction, I should like to outline the principal run of procedures in our laboratory of forensic biology from the point of receiving material to be studied until completion of our expert opinion.

Computer upon receipt by our sub-section shall register any job order. Subdivision proceeds according to the following categories:

1. Comparison between trace and person profiles (investigation ordered by a judge)
2. Drawing up an individual profile for the data base (investigation ordered by a judge)
3. Drawing up a trace profile for the data base (investigation ordered by a judge)
4. Applications for an investigation without a judge's order.

Traces of category four are used for investigations of substances, in case they are traces of blood or traces from cases of sexual abuse (e.g., tests for sperm). All other types of traces from under this category (skin scaling, saliva, faeces, urine etc.) shall be returned unprocessed to the ordering party, complete with a note that investigations may only proceed after a judge's ruling was issued.

Documentation of traces consumes a large share of the processing time under categories one to three. Any object carrying traces shall be exactly described and photographed digitally. Only afterwards the traces will be extracted, tested for substances, and then examined by DNA analysis. Assistants, supported by experts, are responsible for processing a case, beginning with documentation and ending with DNA analysis and they administer the full gamut of technological steps. The experts provide final evaluations of serological findings and of DNA analyses as well as any assessments. The last link of the chain is the expert opinion of the public authority, signed by the expert by order of the director of the State Criminal Investigation Department. This opinion shall be forwarded to the ordering party (ie, the police, the department of public prosecution or a court), and if required, the expert concerned shall represent the findings during the trial process.

Our sub-section deals with traces of criminal offences ranging from damage to property to murder: cases of theft represent the greatest percentage. The following traces are investigated by way of DNA analysis:

Blood:	e.g. clothes, glass or any other objects;
Sperm/Vaginal secretion:	e.g. clothes; vaginal swabs; on sheets and bed covers;
Saliva:	e.g. the filter of cigarettes, food, stamps, openings of cans or bottles or on glasses;
Skin scaling:	both on clothes, on arms or tools to identify the owner, as well as on tools, stones, adhesive tapes, tools used for fettering people or on any other objects to identify the user.

Tissue, faeces and urine.

Hair, bones or any other body fluids not mentioned above shall not be subject to DNA analysis by our own laboratory. For these types of tests we use channels of administrative aid provided by forensic institutes at Greifswald or Rostock as well as by the Federal Office of Criminal Investigation. Our laboratory does not carry out any DNA analyses on animal material either. We are, however, capable of matching animal blood or tissue with an animal species by way of antibody reactions. If a client should insist on any further investigations or tests, however, we shall refer him to private laboratories.

We only run paternity or maternity tests in cases of criminal offences (such as rape).

For the rest of my paper I would like to confine myself to DNA analysis. I will begin with a description of how this type of analysis started in our laboratory and I will then briefly outline the legal context and the nation-wide DNA analysis file (DAD). I will also refer to the present levels of DNA analysis in our sub-section and speak about our prospects.

The beginnings of DNA analysis

Until 1996 investigations in the serology sub-section were confined to customary substance tests, determination of blood groups, tests of serum proteins and enzymes [1].

At that point in time, many biological types of traces (skin epithelium, urine, faeces, saliva and sperm) could not be investigated or any results had only very limited probative strength. Also, much more analysis material was required of all types of traces than is the case for DNA analyses and evaluations today.

In 1995 we began establishing the tools of DNA analysis in the serology sub-section. We decided to use STR-typing [2, p. 746–756; 3, p. 175–189] (short tandem repeat) by polymerise chain reaction (PCR) [4; 5, p. 2–15] methodology as this was also used by all other forensic investigation facilities in Federal Germany. It was also recognised by the courts.

One year later we already used this DNA analysis for the first time in a concrete case, to process DNA results in a serological expert opinion. We were then capable of investigating two autosomal STR systems (F13B and VWA) from the non-encoding genome section. By May 1997 we had enlarged our range of investigations by five more autosomal STR´s (FES/FPS, THO1/TC11, FGA/Fibra, CD4, and D1S80), as well as the sex-determining feature amelogenine. The serology sub-section then consisted of one expert and two technical assistants.

Reaction mixtures for amplifications (multiplications of certain STR´s) were either prepared by our laboratory following instructions published in reference texts, or we used single-plex kits made by Serac (Manfred R. Hoffmann. Serologische Reagenzien GmbH, Bad Homburg Germany). Separation of amplified fragments (STR´s) was achieved by way of a horizontal polyacrylamide gel electrophoresis, followed by silver staining [6] to bring about visibility of alleles (DNA features). Because of the low separations of bands it was very difficult to assess stained gels over a lighted plate.

In 1997 and using this technology, 575 DNA extractions (DNA isolation from traces) were carried out, followed by 2,300 amplifications. So far this year, we already received 600 applications for serological analyses from police offices of Mecklenburg – Western Pomerania. This figure is two times the number of applications received before introducing DNA analyses.

Dispensation of justice follows the development of technology

Although DNA analysis had already been a recognised source of judicial evidence in criminal proceedings following a judgement of the Federal High Court of Justice issued on 21st August 1990 [7], Parliament reacted only in 1997 to regulate the inclusion of molecular genetic investigations into the prosecution of criminal offences [8]. To that end the Code of Criminal Procedure was enlarged by articles 81 e and 81 f, to allow investigations and tests of bodily material of an accused to find out about its origin or to match it to traces found at the scene of a crime. Such material may not be used for any other investigations, such as tests for hereditary diseases. Permission for an investigation granted under these legal regulations also includes trace material found, impounded or confiscated.

The legislator did not draw a line between DNA analyses carried out on the encoding and non-encoding sections of a genome. All the DNA systems investigated by our sub-section are positioned in the non-encoding genome section. The only exception to this is when the sex of an originator of traces shall have to be determined. It is still a matter under dispute whether quoting the sex of an originator of traces is permissible in an expert opinion. So far, however, there has never been any complaint by the courts of Mecklenburg – Western Pomerania.

The new article 81 e does not only allow carrying out investigations into bodily material of accused people, but also into that of other people involved in legal proceedings, e.g. of injured parties, witnesses and others. That means that mass screenings are also allowed in Germany to find an unknown offender.

Legal provisions strictly limit any use of investigated material to the criminal proceedings involved or to any other criminal proceedings pending. Investigated material may only be stored until proceedings are final and absolute; it shall have to be destroyed afterwards.

In order to guarantee a high level of legal certainty for all parties involved in the proceedings, article 81 f lays down that an order requiring molecular genetic investigations must be issued by a judge. Only judges may direct that such molecular genetic test shall be carried out in the context of criminal proceedings. Any such order must be issued in writing and the expert to be commissioned must be quoted by name. Any material to be investigated must be handed over to the expert without mentioning either the name, address, day of birth, or month of birth of any person. Such persons shall be kept totally anonymous.

A large measure of co-ordination was required, involving police authorities, state prosecutors' offices and courts until all organisational aspects matched legal requirements. One limitation was particularly difficult to understand, namely that even a trace left at the scene of a crime could only be subjected to DNA analysis after an order had been issued by a judge. We start our molecular genetic investigations into trace and comparative materials only after all legal requirements have been met.

Provision of a central data base for DNA profiles of criminal offenders and of forensic traces

DNA profiles are (almost) as reliable a feature of individual identification as are finger prints. The idea therefore suggested itself to establish a centralised data pool so as to match traces from the scene of a crime and known criminal offenders.

On 17th April 1998 a central DNA-analysis file (DAD) was established at the Federal Office of Criminal Investigation. DNA profiles of people and of traces found on scenes of crimes are fed into it by the Federal Office of Criminal Investigation as well as by state criminal investigation departments. The inclusion of personal profiles into this data base for people accused in ongoing criminal proceedings is regulated by a new article (81 g) of the Code of Criminal Procedure and by article 2 of the DNA Identification Act (DNA-IFG) [9; 10] for criminal offenders already sentenced previously. Criminal offences must at least be allocated to the medium delinquency range. A descriptive catalogue of criminal offences was attached to this Act. In addition, a "prediction" must be provided, implying there are reasons to assume that a particular person may be involved in renewed criminal proceedings in future.

The legal regulations governing DAD work laid down that only features from the non-encoding part of a genome (some 95 percent of DNA) may be recorded. It is not permitted to record any encoding features (only some five per cent of DNA). Numerical codes are used for recording.

Initially, profiles consisting of five featuring STR-systems each were recorded in the central DNA-analysis file (DAD). Serology experts from the Federal Office of Criminal Investigation as well as of the state criminal investigation departments had agreed on these five systems and used them as core systems for the DAD as of 1997. At that time, four of them had been laid down by the DNA Working Group of the European Network of Forensic Science Institutes (ENFSI) and the European section of Interpol to serve as European standard systems (VWA, TH01, FGA and D21S11). There was also a highly polymorphic – and therefore very meaningful – STR-system called SE 33. It was also laid down that only complete personal data sets, ie, those consisting of all five systems, were to be entered into the DAD. Any trace data sets have to be type-structured under at least three systems and the relative frequency for such data set to occur had to be below one person out of 100,000.

As the data set grew, it was found that there were increasing numbers of "non-scores": person-on-person matches amongst unrelated people.

Another problem resulted from the lack of compatibility of the five German features with the seven European standard systems as now recommended by the DNA working group of ENFSI. As a result, in 2002 the data base was enlarged by three systems (D3S1358, D8S1179 and D18S51) so that is now similar to the European standard for seven STR-systems. At the same time, feature SE 33 continues to be used in Germany as feature number eight in the DAD.

The total number of data sets in the DAD stood at 285,730 at the end of the second quarter of 2003; of these, 245,257 were personal data sets and 40,473 were trace data sets. At that date 3,670 people and 572 traces had been contributed to the DAD files by Mecklenburg – Western Pomerania. Over ninety per cent of those had been processed by our own laboratory.

Almost one out of every five traces newly recorded onto the DAD now leads to a one-person score. In 2002 over 3,300 criminal offences were cleared up by reference to the DAD. Since it has become operational, there were a total of 13,209 scores with validated results by the end of the second quarter of 2003. Of these 9,254 were trace-person matches and 3,955 were trace-trace matches. There is a preponderance of cases of theft (11,690), followed by cases of robbery and blackmail (1,004) amongst scores and matches. There were 537 scores concerning offences against sexual self-determination and 208 matches for crimes against human life. The state of Mecklenburg – Western Pomerania may already boast 82 DAD-related scores, of which 48 are person-trace matches and 34 are trace-trace matches.

This underlines that the DNA analysis file has proven to be a helpful instrument to fight crime already during the first five years of its existence.

Adaptation of laboratory analytical chemistry to data base requirements

We were forced to change our analytical processing methods after the introduction of the DAD file. The separation carried out so far of amplified fragments by way of a horizontal gel electrophoresis with consequent silver staining did not allow for a separation of fragment lengths in the one-base pair section range. Features to be recorded for DAD use, however, require such sub-division. The reason is that, particularly in the SE 33 system, there are allele distances of one base pair.

We decided to switch our technology to capillary electrophoresis [11], then fairly new in DNA analysis and purchased two ABI PRISM® Genetic Analyzers (made by Applied Biosystems, Foster City, USA).

This technology employs fluorescent stains as markers of primers used for amplification. DNA fragments amplified with these primers contain some fluorescent stain at their 5'-end. As a result of their different sizes and charges, these marked fragments will then be separated in a polymer-filled capillary by applying an electric charge. The bigger such a fragment, the more time it needs to reach a window of the capillary. There it will be irradiated with a laser and a fluorescence reaction (a light flash) occurs. This flash shall be registered by a CCD camera in the analyser which converts it into a peak signal. Any sample is equipped with length standards, so that the computer is in a position to calculate base pair lengths of individual fragments and to display them graphically. By referring to so-called allele ladders which include known alleles of individual DNA systems, individual fragments may be named and assigned.

After three months of validation testing we were able to include capillary electrophoresis into our investigation routines. We started with a STR-kit supplied by Applied Biosystems (AB) – the AmpFlSTR Profiler™ Plus – and our own mix of primers with two STR-systems (TH01 and SE 33). As a result and for comparative investigations we had 11 autosomal systems (D3S1358, VWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, TH01 and SE 33) plus the sex-determining feature of amelogenine at our disposal. In Germany the most frequent statistical combination of features for these eleven systems reveals a frequency of one person among about 28 thousand million.

Other STR-kits were also tested over time. For comparative analyses (traces of people suspected of a criminal offence) we now use the AmpFlSTR Profiler™ Plus kit as well as the Power Plex® ES kit made by Promega (Promega Corporation, Madison, USA). We employ the PowerPlex® ES kit for recording sets of data for the DNA file and for personal data sets we make use of the AmpFlSTR SGM Plus together with our TH01/SE 33 combination. That means that apart from eight data base systems we still have an additional six autosomal systems available for routine tests. If found necessary, we are also capable of using another four systems validated by our own laboratory. Altogether then, we may employ eighteen autosomal systems for our case work.

A visibly improved evaluation of trace materials and the enlarged range of traces that may be analysed resulted in a considerable increase of incoming orders. They stood at 600 in 1997 and rose to 1,850 by 2002. Another two ABI PRISM® Genetic Analyzers were purchased and we took on additional staff. Today the serology sub-section consists of two administrative staff, four assistants and two experts.

In 2002 our laboratory carried out about 4,400 DNA extractions, followed by some 11,000 amplifications. Compared to 1997 this is an increase of about 760 per cent for extractions and of ca. 480 per cent for amplifications. To put these figures into their correct perspective, it must be pointed out that in 1997 individual system amplifications were carried out while today up to nine systems may be part of any amplification.

Prospects

Much progress has been observed in forensic DNA analysis over the past few years. New investigative methodology was developed (such as the single nucleotide polymorphism (SNP), biochips); other methods were enlarged or improved upon (DNA analyses of telogenic hair, investigations of minimum traces, Y-STR). Some of these improvements have already been introduced into the work of our laboratory.

Since the beginning of this year we have included Y-chromosomal STR-systems (Y-STR) into routine investigations in cases of sexual offences. As this type of test involves only systems on male Y-chromosomes and a consequent amplification of exclusively male DNA features, such work is of particular relevance in situations where mixed traces of men and women are concerned. Compared to autosomal STR- systems, however, their disadvantage is that they yield a lower probative strength.

Unfortunately we are not yet in a position to carry out investigations into hair, be they morphological or DNA analysis-related. One of the reasons is a shortage of space, but relief is in sight as there are plans to build a new institute in 2006. We shall then integrate shortened primer sequences developed by the Federal Office of Criminal Investigation for DNA analyses of telogenic hair (without roots and with seriously degenerated DNA) into our range of investigations.

We also plan for an extension of minimal trace investigations (skin scalings and other types of traces with low DNA contents). A project group for DNA analyses of problem traces, set up jointly by the state criminal investigation departments and the Federal Office of Criminal Investigation works out test concepts for minimal traces. We hope that these traces may yield significant test results compared to today's outcome. In such cases, present technology only produces partly useful results or others which cannot be evaluated.

If we pursue our continuous modernisation drive of investigation technology and keep it at start-of-the-art levels we also guarantee high-quality DNA analytical investigations of forensic traces for our state police force in future.

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Dabartinė DNR tyrimų būklė bei raidos perspektyvos Meklenburgo-Priešakinės Pomeranijos žemės valstybiniame kriminalinių tyrimų departamente

Dr. Detlef Kauczinski

Meklenburgo-Priešakinės Pomeranijos žemės valstybinio kriminalinio tyrimų biuro departamentas

SANTRAUKA

Remiantis Federalinio Aukščiausiojo Teismo sprendimu dėl DNR tyrimų procedūros, nuo 1996 rugpjūčio Meklenburgo-Priešakinės Pomeranijos žemės valstybinio kriminalinių tyrimų departamento Teismo medicinos instituto Biologinių tyrimų skyrius pradėjo vykdyti pėdsakų DNR tyrimus. Prieš tai tokių tyrimų metodika buvo sukurta ir plėtojama apie metus. Iš pradžių buvo tiriamos tik dvi DNR chromosomų sistemos, o nuo 1997 gegužės buvo įdiegta šešių sistemų analizė. Šiuo metu skyriuje dirba du techniniai asistentai ir vienas ekspertas. 1997 metais buvo paimti 575 pėdsakai bei lyginamieji pavyzdžiai ir atlikti 2277 tyrimai.

1997 metų kovo mėnesį buvo sukurta aiški DNR tyrimų procedūros teisinė bazė, todėl iki dabar teisėjas turi teisę skirti pėdsako, esančio byloje, DNR tyrimą.

1998 metų kovo mėnesį Federaliniame kriminalinių tyrimų biure buvo įsteigta DNR duomenų bazė. Tai pareikalavo ir DNR tyrimų metodikos pokyčių. Dabar mūsų laboratorijoje įmanoma palyginti iki dvylikos DNR sistemų. Nuo 1998 rugpjūčio mėnesio Meklenburgo žemėje 285 000 DNR duomenų buvo įtraukti į visos valstybės DNR duomenų bazę.

Pastaruoju metu galima atlikti šių pėdsakus sudarančių medžiagų DNR tyrimus: kraujo, spermos, seilių, odos epitelio, šlapimo, ekskrementų ir kitų audinių. Yra sukurtos keturios PRISM genetinės analizės ir identifikacijos sistemos. Dabar skyriuje dirba du ekspertai, du padėjėjai ir vienas administratorius. 2002 metais buvo paimta 4400 pėdsakų ir atlikta apie 11 000 tyrimų.

Ateityje planuojama tobulinti plaukų tyrimus, tai turi būti atlikta iki 2006 metų.