

A Review of the Science of Low Template DNA Analysis

Executive Summary

From our detailed review we find that the science supporting the delivery of Low Template DNA (LTDNA) analysis is sound and that the three companies (the Forensic Science Service Ltd, LGC Forensics and Orchid Cellmark Ltd) providing this service to the Criminal Justice System have validated their processes in accord with accepted scientific principles using both 28 and 34 PCR cycles for extracts containing less than 200 picograms (pg) of DNA. At these levels, stochastic and inhibition effects have an impact upon the DNA profiles produced and all those involved in this process have established guidelines for profile interpretation. Work on interpretation is continuing and it is for the Forensic Science Regulator to monitor this and to bring about some standardisation in interpretation amongst all providers.

Now that suitable commercial analytical systems are available for the quantification of low levels of DNA, it becomes important that all DNA samples submitted for analysis under the Criminal Justice System must, as a matter of best practice, be quantified before attempts are made to produce DNA profiles.

Training both laboratory personnel and those involved in the recovery of DNA samples from crime scenes requires to be standardised. It is for the Forensic Science Regulator through a dialogue with all providers and ACPO to establish what those standards should be, to implement them and to monitor their application.

The use of DNA-free consumables both in the laboratory and for those working at the crime scene is essential. Standards for these need to be set and quality control mechanisms put in place to monitor the status of these materials/chemicals.

Although used for a number of years we do not yet have any reliable measure of the success rate of LTDNA analysis and this need to be corrected.

We have been made aware that there is concern by the forensic science providers of the role of Police Laboratories established by the larger police forces. This concern relates to the quality of work and their future role in the provision of forensic science services. Additionally they have expressed concern over the funding of forensic science. It is for the Forensic Science Regulator to enter into a dialogue with ACPO to allay these concerns and /or to develop mechanisms that can overcome any envisaged problems.

We have become aware that there is a desperate need for independent research funding in order to advance the discipline of forensic science. Such funding should be open to all on a competitive basis in the same way as the government funded research councils, who are at present unable or unwilling to finance any developments in this arena.

Finally, we believe that it is important for the Forensic Science Regulator to monitor all documentation associated with accreditation and validation.

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A Review of the Science of Low Template DNA Analysis

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1. Introduction

1.1 Doubts have been expressed about the use of Low Template DNA analyses (LTDNA) for legal purposes (as opposed to the investigating process) and it has been reported that few countries use this process routinely. Since such concerns have been expressed, the Forensic Science Regulator instigated this review and appointed Prof. Brian Caddy of Strathclyde University to chair this process with the support of two DNA experts, Dr Graham Taylor of Cancer Research UK and Dr Adrian Linacre of Strathclyde University.

The terms of reference given to this review panel are:

- i). To examine low template DNA profiling techniques, including the Low Copy Number (LCN) technique employed by the Forensic Science Service Ltd (FSS), and analogous processes used by other providers of DNA profiling services to the UK Criminal Justice System (CJS), to generate DNA profiles from samples which may not yield useable results from the standard DNA profiling (termed SGM Plus® process discussed in section 1.4). This is to include processes which seek to obtain profiles from DNA samples below 200pg and the application of supra-28 cycle amplification;

- ii). To advise upon the scientific validity of those techniques, having regard to any novel issues raised (in comparison with accepted SGM Plus® techniques) and the variations in approach adopted by different providers, recommending best practice in the light of current scientific knowledge and opinion;
- iii). To comment upon the interpretation of the results and how they should be presented to the customer and to the court in any criminal proceedings;
- iv). To advise upon the creation of a national minimum technical standard for low template DNA analysis, to include extraction, quantification/dilution and interpretation criteria; and
- v). To make other relevant recommendations.

1.2 The approach adopted for this review has been to visit the three laboratories that constitute the main providers of forensic science services to the CJS and to speak with personnel involved with LTDNA analysis at both the bench and research levels as well as the management of the laboratories. Additionally, we have visited Harperley Hall (National Policing Improvement Agency Training Centre) to speak with the trainers of crime scene officers involved in the recovery of DNA samples from crime scenes. Furthermore, we have interviewed: a representative of United Kingdom Accreditation Service (UKAS) who are involved in accrediting forensic science laboratory processes, the Custodian of the National DNA Database (NDNAD) and the person responsible for forensic science support at the Metropolitan Police. We have also participated in a forum with senior officers of the Metropolitan police and have corresponded with a representative number of police forces. Our view from the commencement of this review was that for completion it was not sensible to divorce the laboratory processing surrounding LTDNA from those of police activities associated with the recovery of DNA samples. Much of our focus therefore has been on the validity of the scientific processes and the need for instituting strict regimes to eliminate contamination. Forensic science has long been thought of as a process starting at the crime scene and ending

in the court. However the LTDNA process starts beyond the scene and includes the manufacture of items for use at the scene and in the laboratory. There has been insufficient time to make a full comparison of all aspects of LTDNA analysis between all three forensic science providers. For example no direct comparison has been made of their individual extraction processes but since these processes have been well documented and validated for many years when employing standard SGM Plus® systems we do not feel that this is a serious omission. It is assumed that all information provided was accurate, but should further information be made available we are prepared to reconsider our recommendations.

1.3 Dr Peter Gill who was one of the members of staff of the FSS with whom we spoke in relation to this review, has from 1st April 2008, become an employee of the University of Strathclyde.

1.4 With the introduction of the NDNAD, forensic science, as opposed to police science (fingerprints) is, for the first time, able to provide direction to the investigator. The current process of DNA profiling used in the UK examines 10 hypervariable regions of DNA (short tandem repeats or STRs loci) plus a marker to determine gender. This process is termed SGM Plus®. The use of short tandem repeats was introduced in 1994 when 4 loci were analysed, this extended to 6 loci plus the gender test (SGM) and in 1999 the 10 locus test (SGM Plus®) was introduced. These tests were all standardised for 28 cycle amplification from originally 2 nanogram (ng) of DNA before the increase in sensitivity in 1999 permitted 1ng of DNA as the standard starting template.

1.5 The introduction of the polymerase chain reaction (PCR) permits the analysis of tiny bloodstains and other body fluids. The analysis of these PCR products originally took place on gel systems but this latter process has now been superseded by the use of capillary electrophoresis, which produces a series of peaks referred to as a "DNA profile". This process is applied routinely to traces of body fluids that have been identified and recovered from clothing and surfaces and the amplification process in such cases is undertaken 28 times using a pre-prepared SGM Plus® kit. This process has been validated,

is well documented and used routinely both in the United Kingdom and in other countries [1-5].

1.6 The use of 28 cycles was introduced so that STR profiles will be obtained optimally at 1ng of DNA. The commercial kit (SGM Plus®) is optimally designed to amplify a full DNA profile from 1ng of DNA, although DNA profiles of varying quality can be generated from less template DNA. This mass (1ng) of DNA equates to approximately 160 human somatic cells. This number of cells can be visualised as, for example, by a tiny blood spot. The ideal DNA test will detect DNA transferred during an incident but it should be noted that any item examined may have had DNA transferred prior to the alleged incident (considered in this review as incidental DNA). Additionally, DNA may be transferred after the alleged incident (termed contamination DNA). If anti-contamination procedures are used it is aimed that 28 cycles of amplification should not generate full DNA profiles associated with low level contamination.

1.7 Standard DNA profiling which uses 28 cycles works effectively with identifiable traces of body fluids but there are times when no identifiable body fluid is present. The amount of DNA in these samples may be present at very low levels perhaps corresponding to one or more human cells. Some of these samples are sometimes referred to as 'touch DNA' and may be present at levels similar to incidental DNA or that of low level contamination that would not normally be detected using standard DNA profiling. Modifications to obtain an STR profile from less than 200 picogram (pg) include: optimisation of the electrophoresis system, increasing the number of amplification steps from 28 to 34 cycles [6] and/or purification of the PCR product from a 28 cycles process. Any of these modifications results in an increase in the sensitivity of the test but may also increase stochastic effects and the opportunity for detecting DNA not related to the alleged incident (either incidental or due to contamination). The stochastic effects include allelic "drop out", random allelic "drop-in" and an increase in stutter products. These processes confuse the outcome of such DNA profiling and are usually dealt with by repeating the process a small number of times, usually twice is sufficient. The stochastic effects are not limited to increased cycle number but occur even with 28

cycles when using low template DNA. With the introduction of more sensitive systems, e.g. capillary electrophoresis it is possible to detect very low levels of DNA using 28 cycles. Sometimes identifiable cellular material may be present but either the DNA is degraded such that a full DNA profile cannot be obtained, or the presence of inhibitors prevents further analysis. Since the aim is to generate as complete a DNA profile as possible, modifications as described above will be used.

1.8 Additionally, in order to take into account those processes used by some forensic science providers that do not employ additional cycles of amplification to detect very low levels of DNA we have adopted the following definition of LTDNA analysis viz.

“An ultra sensitive technique that has the potential to yield a DNA profile from sub-optimal biological samples e.g. *Low Copy Number DNA analysis*.”

2. Recovery of Samples for Low Template DNA Analysis

2.1 There are two aspects to this process, the first is the recovery of samples, often by Scene of Crime Officers or Crime Scene Investigators (SOCO/CSI) from the crime scene and the second is the recovery of biological samples by the scientists in the laboratory from items submitted.

Recovery of Samples from Crime Scenes

2.2 The first of these rests on the decision of the SOCOs/CSIs often in conjunction with the police Senior Investigating Officer of what samples should be considered for subsequent LTDNA analysis. It is considered important that this type of decision is taken early in an investigation to reduce the possibilities of contamination of such samples. One view expressed to the review is that at this stage of an investigation it is not possible to identify such samples and that in anyway DNA samples would be recovered by a standard procedure irrespective of its importance to the investigation. However, trainers from the FSS have reported confusion amongst some police force scene personnel as to when and how samples for LTDNA are to be recovered. This same problem does not appear to have been encountered by some other

forensic science providers but these have probably undertaken less training and are only recently involved directly with crime scene work. As a consequence we do not entirely support this view but believe that given appropriate training, more focused decisions in respect of recovery of LTDNA samples can be made and any appropriate rigorous decontamination procedures implemented. Where such decisions become difficult, then direct contact with laboratory staff needs to be encouraged. Because of the commercialisation of forensic science provision we are told that some police forces believe that the advice given may be commercially driven and consequently an independent advice service would be looked upon with favour. If this is accepted then the only potential source for such advice may have to come through the National Policing Improvement Agency (NPIA) who would be required to provide trained personnel for this purpose. This suggests that some conformity in approach needs to be established. It is for the Forensic Science Regulator to institute such training programs and mechanisms for the resolution of these issues.

2.3 A most important aspect of this recovery process is associated with the use of DNA free materials particularly in respect of swabs and containers. The FSS do provide these under their SceneSafe Kits especially the K555 kit used to recover 'touch DNA' such as would occur with finger prints. They have entered into agreement with manufacturers to provide such DNA-free materials using a specific ethylene oxide decontamination procedure they have developed. We were informed that batches of these materials are quality controlled (we believe that other forensic science providers should introduce similar controls over their consumables). Additionally they have generated a database of personnel working for the supply companies that can be used for elimination purposes. Such precautions, which the review panel consider valuable, do have a cost implication which may lead some police forces to sanction the use of cheaper substandard kits for such purposes. Other forensic science providers have taken a different view and have put the onus on the manufacturer to provide DNA clean materials. They have encountered some minor problems using this approach leading to "drop in " alleles present in the profiles but these have not been considered a real problem for

interpretation. One of these organisations has looked at the use of ethylene oxide decontamination and believed it to be a promising process. Neither of these organisations batch quality control their consumables nor do they provide police forces with DNA recovery kits. A national standard for “DNA clean” recovery materials needs to be set.

2.4 Some police forces are making use of kits provided by the FSS while others, basically on cost, may choose to go to alternative suppliers. It is not clear how such forces choosing the latter route determine the suitability of these consumables. In all cases surveyed it would seem that wet and dry swabbing are the standard procedure employed. It is for the Forensic Science Regulator in consultation with all parties to establish the standard for DNA-free recovery kits and to make sure that all police forces employ these. Additionally, such kits must be quality controlled.

2.5 Some forces issue detailed instructions on how to recover LTDNA samples while others only give general guidance. There seems to be a feeling in some police forces that LTDNA is a panacea for the solution of all crime because of little understanding of the concepts and making submissions without consideration of contamination issues. Clearly there is a requirement to communicate the limitations of these procedures.

2.6 It should be noted that failure rates for LTDNA analysis are high. One police force has estimated success in achieving a full profile at about 6% although this might be a reflection of the substrates sampled. We have sought detailed information on the success rate of LTDNA analyses but no such information seems to be available. The main reason for this lies in a lack of agreement on what constitutes a success. One consideration may be those situations where standard SGM Plus® has failed to produce any useable profile but that LTDNA analysis has produced a full or useable partial profile. It is for the Forensic Science Regulator to come to an agreement with all parties on what constitutes LTDNA success and to then to institute an appropriate survey.

2.7 Some police forces are aware that because LTDNA analysis cannot often be related to a particular act then this limits its usefulness to an investigation. Other forces are not so aware of the difficulties that arise from LTDNA analyses and an education programme is required to inform and correct any misconceptions. There is some agreement amongst police forces that national guidance on the use of this technique would be useful since even when the scientific advice is that such samples may be better analysed by the standard SGM Plus® system or that the item is unlikely to provide any useful data, some police forces choose to go ahead with such LTDNA analyses. It is for the Forensic Science Regulator to institute appropriate training programs and to set standards that will enable police forces and their crime scene personnel to have a full grasp of what constitutes LTDNA analysis, how such samples are to be collected and stored and the likelihood of success.

Isolation of DNA samples in the Laboratory

2.8 The isolation of DNA from exhibits differs little whether standard or low template DNA samples are being recovered. The main difference is that for the former, automation of the DNA extraction process can be employed while for the latter manual extraction would be more common. Methods for the recovery of DNA from exhibits are well documented using a commercial kit from Qiagen or the phenol/chloroform method and have been validated over many years [7-11].

2.9 In relation to LTDNA analyses it is desirable that a decision is made at an early stage as to whether such a sample submitted to the laboratory would be best analysed by this method. This requires that staff to be suitably trained for this role and we have been advised that in the FSS such specialised training does take place and that the staff are mentored after training and their casework peer reviewed. In addition such staff must undergo a specific written test. We are advised by UKAS that these training programs are of a high standard and that detailed records of conformity to these standards of performance are maintained. For other providers, for this specialised work we were informed that they have recruited personnel who already have the necessary skills although we are aware that at least some of these personnel

transferred to these organisations prior to the introduction of LCN DNA analysis by the FSS. For these organisations training programs comparable to those of the FSS are in place or being put in place for new recruits who do not yet possess the necessary skills. The levels of such competencies will need to be evaluated by UKAS as part of their accreditation process. It is for the Forensic Science Regulator to oversee compliance with these standards of competence for LTDNA specialists and when and where appropriate to suggest modifications to such training programs and record keeping.

2.10 In all laboratories we have visited special precautions have been taken in respect of LTDNA analysis to minimise the risk of contamination. These include pressurised laboratories, ultraviolet irradiation of benches, a strict bench cleaning regime and deep cleaning processes. Contamination levels are continuously monitored. We have observed details of contamination levels and the corrective procedures. All those who work within this environment are suitably dressed with clean overwear, masks, footwear, and double rubber gloves etc. Maintaining such laboratories is expensive and this will be reflected in the cost of providing this service. Some more recent providers of this type of service are fortunate in having newly constructed laboratories of a high standard while the FSS and LGC Forensics has had to adopt much older laboratories and while this does not mean an inferior service it will probably be more costly to maintain.

3. The Analytical Process

3.1 Having recovered the low levels of DNA from exhibits it becomes necessary to submit the sample for analysis. This is a two stage process involving the amplification of DNA from the recovered template DNA using the PCR and finally analysing this product using capillary electrophoresis. In earlier days and at the commencement of the implementation of LTDNA analyses the FSS employed gels for the separation of the DNA products.

Quantification of DNA

3.2 The first question that must be resolved is whether or not the isolated DNA (assumed to be <200pg) should be quantified. Because the amount of DNA is so small the FSS take the view that it is unnecessary to quantify because it uses up too much DNA in the process thereby reducing the chances of producing a successful profile. Other forensic science providers routinely quantify the DNA extract believing that in so doing it reduces the chances of generating over amplification of the PCR products, something the review has observed with some FSS analyses. Additionally these providers believe that this enables a better estimate of potential inhibition. Unless there is a recognised method for addressing problems relating to over amplification, the reviewers would favour this second approach. The availability of real time PCR quantification makes this not only feasible but uses acceptable levels of DNA product leaving sufficient to carry out a full analysis in duplicate with sufficient material being left over for a third analysis should it become necessary. Further research into the best ways of quantifying very small DNA samples (for example using a repetitive DNA target) may be indicated. The Forensic Science Regulator should monitor the use of DNA quantification procedures.

3.3 Two different approaches have been adopted by different forensic science providers to the amplification process but the aim in each case is the same, namely to boost the signal obtained from the PCR product in relation to the background noise. The method adopted by the FSS has been to increase the number of PCR cycles from the standard 28 to 34 thereby producing an increase in the amount of PCR product. From such a process an increase in “drop-out” of the second peak for heterozygote profiles, or difficulty in allocating the second peak, can occur. Additionally stochastic problems become more acute and problems can be encountered with the effects of inhibition but the review has been told by the FSS that these effects can be reduced by dilution. [Rules about inclusiveness have been developed. (See below under interpretation)].

3.4 The method adopted by other forensic providers is to carry out the amplification to 28 cycles and then to submit the product to a “clean-up” procedure prior to analysis by optimised capillary electrophoresis. This has produced profiles that are directly comparable with those of the FSS without requiring additional amplification. In an earlier case, an extract from a tape taken at a crime scene failed to produce a profile of a suspect when analysed by the FSS but that same extract produced a profile when analysed by one of the alternative suppliers in 2004. The reason for this difference in result seems to lie in the effect of excessive DNA inhibition on LCN analyses conducted by the FSS which they did not fully recognise until 2003 and did not correct for by dilution of the sample until 2005. Inhibition of the PCR when using the standard 28 cycles and the SGM Plus® kit was well known in 2001. This is only one example of improved results being obtained from the same sample by two different forensic science providers.

3.5 The way in which scientific methods often develop is that a process will be introduced that seems to meet all validation criteria but that after a period of time using this same process it becomes evident that modifications require to be instituted to optimise the system. This appears to have been the case with the FSS LCN DNA system when it was discovered in 2003 that inhibition processes were preventing DNA profiles being obtained from some samples. Their solution to this problem was to dilute the samples thereby diluting the inhibiting factor, including the amount of DNA, and enabling a profile to be obtained. This discovery meant that the FSS were required to re-test some 5,000 samples. The Reviewers note that this was a failure on behalf of the FSS to inform the police forces of this finding and the length of time, until September 2005, the dilution process took to implement. Moreover, since inhibition was at the heart of the problem and one aspect of this was the presence of excessive amounts of DNA, this should have triggered the thought processes into implementing some quantification of the extracted DNA prior to amplification and analysis. However, it was after July 2005 that a reliable quantification method became available. Prior to this only a rough guide as to the amounts of DNA in an extract was obtained from a measurement of the intensity of a fluorescent dye binding to the DNA

fragments. In some cases this may have been sufficient to evaluate the presence of excessive amounts of DNA and enabled appropriate dilutions to have been made. Unlike all other providers the FSS do not, even now, routinely quantify their DNA extracts. The Forensic Science Regulator should insist that as a matter of best practice a DNA quantification step is implemented for all DNA analyses submitted to the CJS and should monitor its implementation.

3.6 A valuable test would be for a direct comparison study of the two different approaches to LTDNA analyses to be undertaken. The Review has been informed that a scientific paper by one of the forensic science providers describing this is expected to be published soon. Whether or not this scientific paper is published the Forensic Science Regulator should provide a mechanism that, while retaining appropriate confidentiality for the research, he is able to evaluate the information for all forensic science providers and thereafter take appropriate decisions as to best practice. All providers agree that regardless of which signal enhancement method is selected, the problems of allelic drop out due to stochastic effects in the presence of low quantities of template and that of increased noise will occur in sub-optimal DNA samples.

Interpretation of LTDNA Profiles

3.7 An ideal profile, such as could be obtained from fresh blood or a buccal sample, would contain alleles for each of the loci in the test kit. LCN-like approaches deal with minute samples of DNA. These may also be contaminated with inhibitors of the PCR, extraneous DNA and be below the threshold of detection by PCR. All approaches to increasing the sensitivity of detection face the same problems: loss of alleles by chance because they are not amplified from minute starting quantities (allele drop out); high cycle numbers may increase the risk of polymerase induced “stutter-bands” leading to additional alleles and detection of low levels of extraneous contamination.

3.8 In summary therefore the main problems arising from LTDNA profiles reside in inhibition, the “drop out and in” of different alleles, an increase in the

number of irrelevant alleles detected and mixture interpretation. Inhibition is well known to occur with some dyes e.g. from blue denim garments, but there may be other inhibitors including chelating agents, organic contaminants like haem, and heparin that are difficult to remove [12]. Excessive DNA may also affect the interpretation of the resulting DNA profile and this is not restricted to LTDNA.

3.9 In respect of “drop-in” alleles these are considered a random and infrequent process associated with contamination especially of the consumables but also arising from crime scene and laboratory personnel. While laboratory personnel can usually be eliminated from a DNA profile fairly quickly, the incomplete nature of the Police Elimination DNA database is a problem and the Forensic Science Regulator needs to pursue this problem with ACPO. “Drop-in” alleles can sometimes be explained by reference to a manufacturer’s and laboratory personnel database, but may also be helped by employing assured DNA free consumables and reagents. “Drop-out” alleles are also a problem and rules have been developed by the FSS following a study of many profiles under standard but not LTDNA profiling conditions [13, 14]. Other providers have developed similar guidelines for the interpretation of potential allelic “drop-out” and “drop-in”.

3.10 Efforts have been made to develop a consensus for the interpretation of STR profiles from low template DNA [15-17]. Peak areas are normally measured in relative fluorescence units (rfu’s) and it has been reported that provided that the weaker of two peaks for a heterozygote is 60% or greater of the larger peak then it should be included in the profile. Using 34 cycles, where the relative fluorescent units are greater than 10,000 and there is no other detectable allele, then this should be considered a homozygote by the FSS, although this view is not accepted by all the providers. These data are based upon 34 cycle amplification by the FSS and not using the methods adopted by the other two providers.

3.11 All providers agree that regardless of which signal enhancement method is selected, the problems of allele drop out due to stochastic effects in the

presence of low quantities of template and that of increases noise will occur in sub-optimal DNA samples. The recent publication [18] also contributes to development of quality guidelines. The peer reviewed scientific paper discussed in the Omagh Bombing case (The Queen v Sean Hoey (Belfast Crown Court 2006) [19] provides a mathematical model designed to predict the detection of alleles under “normal” and low concentration conditions. Whilst the paper is insightful it cannot be regarded as definitive and the work needs replication by an independent group before it can be accepted. It seems, therefore, that the current state of the art with respect to the analysis of low concentration DNA STR profiles is not yet represented by a legal and scientific consensus, regardless of the quality of the data presented in a particular case. The lack of clear, explicit consensus reflects the extremely challenging nature of the analysis. At the same time, it is clear that the need to articulate such a consensus at national and ideally at international level is pressing. The recent publication [18] is a step in the right direction. As it stands, this represents an agreement from the providers themselves. It does not include a “user” perspective and needs to actively take account of legal as well as scientific arguments. Furthermore, it does not go into sufficient detail regarding criteria for data acceptability or how these are achieved.

3.12 The Forensic Science Regulator should develop a consensus from all the forensic science providers in consultation with all stakeholders on how profiles and mixed profiles are to be interpreted. Once these criteria have been agreed then the Regulator should monitor their implementation.

Validation

3.13 A key question is whether or not the process(es) involved in LTDNA analyses have been adequately validated and is such a validation accepted by the international forensic science community. The question that arises from this is “What is to be expected of a validation process?”. Mr Justice Weir, the judge in the Omagh Bombing case (The Queen v Sean Hoey (Belfast Crown Court 2006), made some useful comments concerning validation. He said:

“Validation is the process whereby the scientific community acquires the necessary information to:

- *Assess the ability of a procedure to obtain reliable results*
- *Determine the conditions under which such results can be obtained*
- *Define the limitations of the procedure*

The validation process identifies aspects of a procedure that are critical and must be carefully controlled”

Mr Justice Weir went on to say that:

“The absence of an agreed protocol for the validation of scientific techniques prior to their being admitted in court is entirely unsatisfactory”

3.14 The Reviewers are entirely in agreement with these statements and seek to assess how far the providers of LTDNA analyses comply with these. Because science is fundamentally an exoteric process, it is the norm in empirical science that findings and data are independently replicated prior to widespread acceptance. Lack of refutation is not sufficient of itself, regardless of the source of the original work. The lack of a funding mechanism to enable this type of scientific enquiry is a barrier to the process of validation of new approaches. The Forensic Science Regulator should seek funding for independent research and validation that is open to national competition.

3.15 To provide validation it is normal practice to begin with samples of known provenance and to submit them to the process and then to see how they comply with the expected outcome. This latter may require a statistical evaluation. For the LTDNA analyses the parallel would be the taking of a large number of DNA samples of known profiles reflecting the different alleles at the 10 different loci. These samples would then be serially diluted to provide masses of say 10, 20, 40, 80, 100 and 200pg. Each of these would then be submitted to the extraction procedure, appropriate PCR regime followed by capillary electrophoresis. The profiles so produced would then be compared with the known profiles of these samples and an evaluation made in respect of

all the parameters discussed above. This process should then be repeated enough times to obtain a statistically robust measure of the reproducibility of the system. This would address the well-founded criticism of Mr Justice Weir who commented on how many times the analysis should be performed and who was not convinced by the response of duplicate analyses when it was demonstrated that a third analysis had provided a 'different' profile. In this context "the same" does not mean identical since not all alleles may be amplified in the replication process. Casework restrictions in terms of sample availability will always have to be taken into consideration. Similarly, mixtures of two profiles (or more) at these same mass levels could be constituted, extracted, analysed and evaluated.

3.16 The review is in receipt of comprehensive documentation from Orchid Cellmark Ltd and LGC Forensics that relates to their validation processes for LTDNA analyses systems Enhance and DNA senCE respectively. These systems employ 28 cycle PCR followed by a clean-up procedure prior to capillary electrophoresis. This documentation sets out a logical progression of experiments to determine the limitations of their processes, these include, optimisation of the capillary electrophoresis system, limits of detection, the reproducibility and evaluation of stochastic effects, drop in and out and inhibition in a way very similar to the pattern of experiments the review advocates above. These experiments represent a true internal validation which UKAS have accepted as complying with ISO 17025. Additionally, these organisations have effectively validated, in a similar way, LCN using 34 cycles as used by the FSS. These last experiments serve as independent confirmation of the system employed by the FSS, although it must be recognised that the FSS did not quantify the DNA prior to analysis.

3.17 The FSS initially provided the review with documentation in support of a validation process from the early years of LTDNA analyses but this documentation was mainly centred on published scientific papers especially the papers of Gill et al [16] and Whittaker et al [20]. This documentation did not follow what we would consider the normal approach to a validation process such as that submitted by the other forensic science providers but we

were assured by the representative of UKAS that in order to comply with ISO 17025, the FSS must have provided them in 2000 with material of the kind expected and as the use of the technique developed, additional criteria would have had to have been met. In order to assess the validity of these publications we have examined in detail a sample of the raw experimental data associated with this work. While always responding to our requests for information in a timely manner the FSS found difficulties, even after providing the validation model illustrated above, in providing what was required. We eventually received from the FSS experimental data setting out a validation process comparable to the suggested model and including reproducibility information, part of which is from a previous publication [16] and some of which appears as an in-house study. In our opinion, collectively the documentation and data provided represent a validation of LCN DNA analyses. We find this inability to respond quickly to our original requests difficult to rationalise because we were assured by UKAS that copies of the validation procedure accepted by them (UKAS) as conforming to ISO 17025 should always be kept by the accredited laboratory and should be readily available for inspection. The fact that all three organisations have independently demonstrated the viability of LTDNA analyses employing 34 cycles demonstrates that this technique is fit for purpose although the other two providers use this process as a last resort preferring their own modified 28 cycle systems. The FSS has undertaken to develop and align its validation procedures with the recommendations of ENFSI and SWGDAM. The Forensic Science Regulator should institute a regular program of inspections of documentation associated with all validations.

3.18 We note that an additional validation process was undertaken in 2007 by Orchid Cellmark following developed validation studies over the period 2000 to 2005. LGC-Forensics validation on the 28 cycle (DNA SenCE) was from 2001 onwards and the FSS validation studies were conducted from 1999 onwards.

3.19 There is not currently one standard method employed by these three organisations to achieve DNA typing of these sub-optimal DNA templates.

The term LCN was one adopted by the FSS for their own methodology but any differentiation from this process, such as that conducted by the other two forensic providers, can also be termed low template DNA typing (LTDNA).

3.20 External validation can only be achieved if the process is accepted by the wider scientific community. The International Society for Forensic Genetics has published guidelines for sub-optimal DNA testing [21]. The use of LTDNA in the international forensic science community has been limited until recent times with only the main laboratory in the Netherlands (NFI) having implemented the LCN 34 cycle DNA typing method in Europe. Other forensic science providers who use 34 cycles routinely are: Environmental Science and Research Ltd (ESR) in New Zealand and the Office's of the Chief Medical Officer in Switzerland and New York State. Similar systems of amplification of sub-optimal DNA operate in Bosnia-Herzegovina, Spain, Italy and Germany. The review is in possession of details provided by the Dutch laboratory which demonstrate an independent validation of the LCN DNA process similar to that implemented by the FSS. This process is accepted by the Dutch Criminal Justice System. Such a validation shows international acceptance of the principal of using 34 cycle processes. Additionally, while some countries do not operate such a system of analysis, LTDNA evidence is accepted in their courts.

3.21 International acceptance requires the implementation of the technique by countries other than the United Kingdom. Some of those countries who have presently implemented such a scheme as detailed above, and while not large in number, supports limited international recognition. This does not in itself negate the technique as being scientifically justified and as being used in the United Kingdom CJS. Scientific papers [17, 21] provided by the FSS indicate that a consensus accepting the general process leading to profiles generated by a validated LTDNA regime has been accepted by the international community such as ENFSI and the European DNA Profiling Group (EDNAP) but these agreements do not provide experimental details of the initial validation process. Nor is it clear if or how they address alternative proposals for dealing with incomplete data.

4. Transfer of Cellular Material

4.1 Research has discovered that individuals vary in their propensity to transfer their cellular material to an object. Further factors that affect the transfer of cellular material include time and pressure. Transfer of cellular material from a person to an object is termed primary transfer. Once transferred the cellular material may be transferred to another surface if direct contact is made between these two surfaces. This secondary transfer is dependent upon the same factors of time and pressure. Additionally the nature of the two surfaces will affect whether cells transfer readily or remain predominantly on the primary surface. Transfer from a primary surface to a secondary surface will not be 100% and therefore there will be less cellular material transferred to the secondary surface than that on the primary.

4.2 There was a caveat in the LCN DNA profiling reports by the FSS which stated that any cellular material generating a result using LCN may have transferred by means unknown. This caveat no longer seems to be stated in recent witness statements relating to touch DNA. LTDNA is used on bone samples, teeth and material removed from microscope slides, in which case the cell type is either known or inferred. There may be good reason for this omission arising from additional research, but any such work has not been made available to the Reviewers. The Forensic Science Regulator should encourage openness in the availability of information that may have an impact on the way DNA profiles are interpreted in the context of a case.

5. Persistence of Cellular Material

5.1 Once cellular material has been transferred from an individual to an inert surface the cellular material will start to be degraded by cellular enzymatic actions. The speed of this degradation is affected by temperature and the presence of moisture. Due to the large number of factors that affect the degradation of cellular material, and in turn DNA, it is unusual to be able to comment upon the time that the cellular material was deposited unless the cells are associated with a particular stain type. The nature and reason for

using LCN precludes such association unless the DNA can be associated with discrete items such as bones, teeth and degraded bloodstains.

6. The Omagh Bombing Trial

6.1 Following the initiation of this review by the Forensic Science Regulator based upon a high profile case, it became known that there was likely to be difficulties associated with LTDNA analyses in respect of the Omagh Bombing case. The reviewers felt it desirable to read the transcripts of the trial in order to evaluate whether or not additional problems associated with the use of LTDNA could be identified.

6.2 Much of the focus of the trial was on the validity of LCN DNA analyses as a technique and as to whether or not the process had been properly validated both internally and externally. The approach that seems to have been adopted by the FSS as described in court was to see the LCN technique as an extension of the established and internationally validated standard procedure using 28 cycle PCR methodology. This validated procedure was then extended by two main publications in Forensic Science International (15,16). It was declared that LCN was a technique that was “reliable, robust, reproducible and fit for purpose”.

6.3 The validation was severely criticised by the defence in terms of reproducibility, lack of quantification, interpretation and the establishment of guidelines. These areas of criticism have been dealt with in discussions detailed above. Contamination was also an issue especially any arising from imperfect packaging and storage. While in the laboratory contamination was under control, the FSS accepted that insecure packaging could lead to contamination problems in relation to traces of DNA.

6.4 Since the judicial decision in this case the FSS have presented additional experimental data to the review and we have accepted these as supporting a proper validation of this methodology. The Forensic Regulator will be best placed to review any future validation studies.

7. LCN in the Criminal Justice System

7.1 The increase in the cycle number from 28 to 34 as employed by the FSS, or the 28 cycle plus clean-up as used by other providers has led to the opportunity to obtain DNA profiles that may not otherwise be generated. With the increase in the sensitivity of the methods come additional interpretation problems and stochastic effects.

7.2 Since the introduction of LCN DNA profiling by the FSS in 1999 to the CJS there had been limited challenge to the process in the UK until the case of R v Hoey (Belfast Crown Court, 2006). Questions were raised by Honourable Justice Weir as to the validity and reliability of the LCN technique. It is our opinion that LCN and LTD are extensions of the internationally accepted process of standard DNA profiling. Since 1999 there have been a number of advances that have increased the sensitivity of DNA testing such that full DNA profiles using SGM Plus® are possible from less starting material. One consequence has been the increase in the number of mixed DNA profiles and low level minor profiles from cellular material that may not have been detected previously. The forensic science community in the UK has developed interpretation guidelines to assist with these types of cases [18, 21].

7.3 Initially it was our opinion that there remained some ambiguity as to the extent of validation conducted by the FSS with regard to the LCN technique. These reservations have been allayed from a study of the raw data produced by the FSS that has been provided to the review, recent experimental work conducted by the FSS and also from detailed information submitted by the other forensic science providers which clearly demonstrate the soundness of LTDNA analysis (including LCN) providing all the appropriate conditions are met. It appears that it is the position of the FSS and the UK Accreditation Service that the technique of LCN is fit for purpose and we would wish to support this view. The LTDNA/LCN DNA technique conversely has yet to be implemented widely by the international forensic science community.

7.4 It is our opinion that any LTDNA profile should always be reported to the jury with the caveats: that the nature of the original starting material is

unknown; that the time at which the DNA was transferred cannot be inferred; and that the opportunity for secondary transfer is increased in comparison to standard DNA profiling. There may perhaps be some exceptions (see section 4.2 above).

7.5 It is our opinion that when DNA profiles match as a result of LCN DNA profiling, the significance of the match should be reported on the probability that the two DNA profiles match only [6]. As the results were obtained from LCN it is inappropriate to comment upon the cellular material from which the DNA arose or the activity by which the DNA was transferred.

8. General Discussion

8.1 Forensic DNA analysis is a huge asset to national and international law enforcement, yet at the extremes of its applications there are limitations and the boundaries of these limits are now under critical review. This is entirely appropriate and timely. It should not be regarded as a threat to service delivery and does not undermine the basic principles of forensic DNA analysis. Nevertheless the challenges in terms of statistical interpretation of the data and in communicating them to a largely innumerate criminal justice system should not be under-estimated, nor should the importance of earning and maintaining public confidence in the system. This will be best achieved by encouraging open review, debate and investigation of the process.

8.2 It is not our purpose to be too prescriptive in our recommendations but rather advisory since it is important that this review does not restrict innovation but encourages research and further developments. To this end it is important that a pool of research funding, open to all by direct competition, is made available since at present such funding is not accessible through normal government funded science research bodies.

8.3 All forensic science providers have expressed to us their concern over the quality of police forensic science laboratories established by the larger police forces as a financial saving and used for screening of items to establish what to submit to the established forensic science providers. Such laboratories

have implications for contamination issues relating to all types of trace evidence, including DNA. They also present problems for interpretation when only selections from specific exhibits are submitted to forensic science providers. Under these conditions no-one has a complete overview of a case and this can lead to future problems. Finally, such laboratories do not operate a properly constituted Quality Assurance system and are not accredited to ISO 17025 standards but should be. This position needs to be remedied. The Forensic Science Regulator needs to enter into a dialogue with ACPO as to the way such laboratories are to be integrated into the scheme of forensic science provision.

8.4 One forensic provider commented that, in their opinion, some police forces do not allocate sufficient resources to ensure the delivery of high quality forensic services. It was suggested that, if this is a problem, spending on forensic services should be ring-fenced. The Forensic Science Regulator may wish to explore the validity of this comment, although we understand that the overall spend on forensic services does already account for a sizeable proportion of a force's budget and that, as is the nature of such things, a force's spend on forensic services does have to compete with spending on other policing priorities. Furthermore, increased competition in the procurement of forensic services should help to ensure that police forces obtain additional value for money.

9. Conclusions

9.1 DNA profiling using STR variants is a well established and robust method. Low template DNA profiling techniques pose additional challenges for data analysis in terms of allele dropout, band stutter and allele gains. Many services have moved into the low template domain by dint of improved technology such as capillary electrophoresis without necessarily being explicit that this is the case.

9.2 The scientific validation of the approach is complex and still being developed. This needs to be continued with the explicit goal of delivering clear, easily understandable guidelines for 9.3) and 9.4) below. It is however,

our belief that the organisations providing such a DNA service in terms of LT DNA analysis have complied with a normal in house validation process based upon sound scientific principles. This has been seen by UKAS as complying with the ISO 17025 standard and we would endorse this. For completion, we would normally wish to see such validation independently ratified by another such laboratory to conform to normal scientific practice of repeatability by an independent laboratory. The commercialisation of forensic science makes this very difficult and it is only through this review that we have seen that this has been effectively achieved by the FSS's commercial rivals. This should perhaps be a driver for the establishment of a professional organisation of all forensic science providers where cross validation can be discussed. The Forensic Science Regulator should explore the means of establishing a professional forensic science provider's organisation in order to develop mutually agreed standards.

9.3 Interpretation of the results is complex for two reasons: the statistics are challenging and probably hard to comprehend by a non-specialist and the decision how and when to apply certain statistical methods has not yet reached a clear consensus, such that the methods applied by laboratory "a" can be directly compared to the methods applied by laboratory "b", e.g. New York and UK laboratories. This is bound to create confusion in a court setting and a clear protocol to avoid this confusion is required.

9.4 National minimum technical standards for extraction, quantification/dilution and interpretation criteria need to be agreed by not only the providers, NDNADB and UKAS but also by users including the police and the criminal justice system. As the providers have made significant progress in this direction already, it may be possible to achieve this in a few months. This would be a highly desirable outcome. The Forensic Science Regulator needs to coordinate all the information already available that is associated with these techniques and by agreement with all stakeholders establish appropriate standards.

9.5 DNA technology continues to advance rapidly and there needs to be a mechanism for horizon scanning and for the funding of ambitious, service-

driven scientific and technical development to enable the UK to maintain its pre-eminent position in forensic DNA analysis.

10. Recommendations

1. For SOCOs/CSIs and SIOs, there needs to be a national education programme setting out the advantages and limitations of LTDNA in order to establish a conformity of approach to crime scene work. From this should be developed national guideline documentation. It is for the Forensic Science Regulator to institute such training programs and mechanisms for the resolution of these issues.
2. It is for the Forensic Science Regulator to come to an agreement with all parties on what constitutes LTDNA success and to then to institute an appropriate survey.
3. It is for the Forensic Science Regulator to institute appropriate training programs and to set standards that will enable police forces and their crime scene personnel to have a full grasp of what constitutes LTDNA analysis, how such samples are to be collected and stored especially in relation to issues of contamination and the likelihood of success.
4. The Forensic Science Regulator should monitor the use of DNA quantification procedures.
5. We have been told that there is an urgent need for the DNA profiles of all serving operational police officers and crime scene personnel to be included on the Police Elimination Database and for forensic science providers to have direct access to it as a means of eliminating irrelevant DNA profiles. While laboratory personnel can usually be eliminated from a DNA profile fairly quickly, the incomplete nature of

the Police Forces DNA database is a hindrance and the Forensic Science Regulator needs to pursue this problem with ACPO. As an alternative, financial support needs to be provided to enable the DNA profiles of Police Officers and crime scene personnel involved in a specific investigation to be obtained at the same time as the suspect samples.

6. A national standard needs to be established for “DNA clean” consumables, especially in relation to crime scene recovery kits. The Forensic Science Regulator should ensure that only kits which meet such a standard should be used by police forces.
7. The Forensic Science Regulator should ensure the batch testing of all DNA reagents to ensure that they are DNA free prior to their use.
8. It is for the Forensic Science Regulator to oversee compliance with standards of competence for LTDNA laboratory specialists and when and where appropriate to suggest modifications to such training programs and record keeping.
9. Those police forces that have made the decision to carry out preliminary forensic testing by the establishment of a police forensic science laboratory must have such laboratories accredited to a standard comparable to those of forensic science providers and should comply with ISO 17025 through UKAS. The Forensic Science Regulator needs to enter into a dialogue with ACPO as to the way Police laboratories are to be integrated into the scheme of forensic science provision.
10. For all LTDNA samples and taking into account the limitations of the amount of DNA extracted from crime samples, quantification of the material extracted for analysis must be undertaken. Satisfactory commercial kits are now available for this purpose. Further research is required into the best ways of quantifying very small samples of DNA

such as using repetitive DNA target. The Forensic Science Regulator must insist that as a matter of best practice a DNA quantification step is implemented for all DNA analyses submitted to the CJS and should monitor its implementation.

11. There needs to be a national agreement on how LTDNA profiles are to be interpreted especially in relation to “allele drop in and out”, stochastic effects, inhibition, and mixtures. This should be aided by regular circulation of appropriate test profiles and interpretation by ALL providers of this service and any results should be coordinated through the forensic science regulator. The Forensic Science Regulator should develop a consensus from all the forensic science providers in consultation with all stakeholders on how profiles and mixed profiles are to be interpreted. Once these criteria/standards have been agreed then the regulator should monitor their implementation. The Forensic Science Regulator should encourage openness in the availability of information that may have an impact on the way DNA profiles are interpreted in the context of a case.
12. The Forensic Science Regulator should institute a regular program of inspections of documentation associated with all validations.
13. Appropriate caveats should be stated in witness statements/court reports, in most instances, when LTDNA analyses have been undertaken.
14. Any new methods of analysis used by a forensic science provider that will result in the presentation of evidence to the courts must be validated using appropriate and sound internationally recognised scientific principles. The details of such validation, including copies of raw data, should be lodged with the forensic science regulator before it is introduced into service. At least once a year or when the regulator decides it appropriate, such validations will be reviewed by an

independent internationally recognised expert panel the composition of which will be determined by the regulator.

15. An independent study should be undertaken to assess the advantages and disadvantages of the two different approaches to LTDNA analysis. The Review has been informed that a scientific paper by one of the forensic science providers describing this is expected to be published soon. Whether or not this scientific paper is published the Forensic Science Regulator should provide a mechanism that, while retaining appropriate confidentiality for the researchers, enables other providers to evaluate the research.
16. Improve existing guidelines and standards. Active development of a consensus approach to the analysis of partial or contaminated DNA profiles is already underway and needs further work and an inclusive structure that takes account of all of the stakeholders. National and international providers have led the way, but this now needs to include close consultation with users. Within the UK a steering or advisory group comprising providers, users and independent legal advice, with perhaps lay representation should endeavour to develop documentation that would guide the courts in the interpretation of evidence. Educationalists and users should evaluate its comprehensibility and review it in a timely manner in the light of legal precedent and scientific advances.
17. The use of STR's clearly has limitations and is essentially 15-year-old technology. The world of genetics and genome analysis has moved on a great deal since then and there may be benefits to be had from alternative technologies. For example, the world of forensic archaeology has been transformed by the use of next generation sequencers [22, 23] and it seems likely that these could have a huge impact on forensic DNA analysis. Furthermore PCR and other amplification technology has improved, with the development of emulsion PCR [24, 25] that may have substantial advantages and

enable backward compatibility with the existing STR database. At present any service developments would have to be recovered as a service cost overhead. This would preclude radical advances requiring substantial funding. These developments may require high-level academic input and a competitive funding mechanism similar to those used by the Research Councils. Opportunities to tap into the international expertise of the Wellcome Trust Sanger Institute, a world leader in DNA sequencing technology, could be investigated. A UK working group focussed on such developments should develop an option appraisal. The risk of not doing this is the stagnation and decline of standards on forensic DNA analysis, whereas a successful programme would secure a world lead for the UK.

18. An open funding mechanism needs to be put in place that will support an independent validation process of new developments. The Forensic Science Regulator should seek funding for independent research and validation that is open to national competition.
19. National minimum technical standards for extraction, quantification/dilution and interpretation criteria need to be agreed by all forensic science providers. These standards should also be agreed by the Forensic Regulator's Forensic Science Advisory Council. The Forensic Science Regulator needs to coordinate all the information already available that is associated with extraction etc. techniques and by agreement with all stakeholders establish appropriate standards.
20. Since this matter of financing forensic science has been brought to our attention by the forensic science providers we believe it is for the Forensic Science Regulator to quantify this problem and to explore mechanisms to correct any problems his inquiry may reveal.
21. The Forensic Science Regulator should explore the means of establishing a professional forensic science provider's organisation in order to develop mutually agreed standards.

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13. Glossary

ACPO	The Association of Chief Police Officers of England Wales and Northern Ireland
Allele	One of two or more alternative forms of a gene or marker such as an STR.
Allelic "drop-in"	Refers to an apparently spurious allele seen in electrophoresis, potentially giving a false positive for that allele.
Allelic "drop-out"	Refers to an allele that should be present, but is not detected by the particular test, effectively giving a false negative for that allele.
Capillary Electrophoresis	Electrophoresis utilising a narrow polymer filled tube to perform DNA size separation.
CSI	Crime Scene Investigator; more commonly called a Scenes Of Crime Officer.
DNA	Deoxyribonucleic acid (DNA) contains the genetic instructions used in the development and functioning of all known living organisms and some viruses.
DNA Profile	A DNA profile is made up of target regions of DNA codified by the number of STR repeats at each locus, SGM Plus® targets ten STR loci plus the gender marker Amelogenin.
EDNAP	The European DNA Profiling Group which is made up of representatives from member countries to assist in developing common standards for DNA analysis in forensic casework.
Electrophoresis	A method of separating electrically charged molecules typically by drawing them through a medium such as a gel under the influence of an electrical potential difference.
ENFSI	European Network of Forensic Science Institutes.
ESR	Environmental Science & Research (Institute of Environmental Science & Research Limited) is a limited liability company, wholly owned by the New Zealand Government.
Harperley Hall	The NPIA National Training Centre in Durham for scenes of crime training.
Heterozygote	Refers to two different alleles being present at one locus resulting in two peaks after electrophoresis, where as a homozygote has two identical alleles which would be observed as a single peak at that locus.

LTDNA	Low Template DNA
Locus (<i>plural</i> Loci)	Specific regions on a chromosome where a gene, or in this context an STR resides
NDNAD	The National DNA Database, the custodianship being with the National Policing Improvement Agency (NPIA).
Nanogram (ng)	A nanogram is 10^{-9} or one thousand millionth of a gram.
NPIA	National Policing Improvement Agency; it has a wide remit including the NDNAD and specialist training of police staff in forensic science.
PCR	Polymerase Chain Reaction is an amplification process that yields millions of copies of the targeted DNA fragment through repeated cycling of a reaction involving DNA polymerase. In each cycle the amount of target DNA is approximately doubled.
Picogram (pg)	A picogram is 10^{-12} or a million millionth of a gram.
Polymerase	A polymerase is a naturally occurring enzyme that catalyses the formation and repair of DNA.
SceneSafe [®]	A company within Forensic Science Service Ltd.
SGM Plus [®]	Refers to the AmpF ϕ STR [®] SGM Plus [®] PCR Amplification Kit from Applied Biosystems used to generate DNA profiles for the NDNAD.
SIO	Senior Investigating Officer.
SOCO	Scenes Of Crime Officer also known as a Crime Scene Investigator in some forces.
Somatic Cells	Cells forming the body of the organism.
Stochastic	Statistically random.
STR	A short tandem repeat, typically where a part of the DNA molecule, comprising a 2-10 base nucleotide sequence, repeats.
Stutter products	Amplification products arising from strand slippage during the PCR, typically seen as a peak one repeat unit less in size than the true allele after electrophoresis normally less than 15% of the area of the major peak.
SWGDM	The Scientific Working Group on DNA Analysis Methods is FBI sponsored group in the USA.
UKAS	The United Kingdom Accreditation Service.