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THE RELIABILITY OF TRACE DNA OR LOW COPY NUMBER (LCN) DNA EVIDENCE IN COURT PROCEEDINGS*

SUMMARY

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Although forensic DNA testing is well established, some experts disagree with the interpretation and statistical significance of test results obtained from very small samples. This article discusses the problems regarding the use of the low copy number (LCN) technique as well as the value that can be derived from such an analysis. It focuses on the problematic results that can arise from using very small samples for forensic DNA identification. Since this kind of analysis is based on low amounts of DNA samples (between 100 picograms and 200 picograms in South Africa) that are amplified by using more than the normal 28 cycles to create larger samples for analysis, the reliability of the analysis has been questioned. The amplification process, known as the Polymerase Chain Reaction (PCR), is associated with risks such as stochastic effects and contamination that could make interpretation of the results difficult for the defence. While standard operating laboratory protocols could prevent contamination and although the electropherograms could aid the detection of contamination, it is highly problematic for the defence counsel to ascertain whether these procedures were indeed strictly followed. Drawing on foreign jurisprudence, this article considers the risks and key controversies and explains what lawyers need to know, in order to be able to recognise controversial results that could stem from using the LCN DNA technique for forensic DNA identification. The conclusions thus drawn may be of particular relevance to the South African context, as no reported case law exists in which the issues relating to the use of LCN DNA have yet come to the fore.

1. INTRODUCTION

1.1 The exponential application of DNA test results in legal proceedings

Since the first case using DNA forensic testing approximately 35 years ago,¹ testing low template DNA



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¹ Gill *et al.* 1989:577-579.

“trace or touch” samples have become possible in recent years as a result of an increase in DNA detection sensitivity.² Initially, forensic scientists required large amounts of genetic material to produce a DNA profile. However, nowadays, investigators can retrieve DNA profiles from as little as three skin cells left behind when a criminal handles a gun, grips a steering wheel, turns a doorknob, or throws a brick.³ By merely touching a surface, a perpetrator can leave behind trace amounts of skin or epithelial cells that are invisible to the human eye. Scientists can work with these low template smaller DNA samples by usually amplifying them at least 32 times instead of 28 times, as is the case in the ordinary Polymerase Chain Reaction (PCR).⁴

Nevertheless, the interpretation of trace DNA, also known as low copy number DNA, such as DNA deposited by touching an object or a person, is far more complex and problematic than that of the relatively large samples previously used.⁵ The complexity regarding the analysis and interpretation of DNA traces, cautions legal fact finders not, inappropriately, to attach more weight to such DNA evidence than to the other available evidence.⁶

Low copy number (LCN) testing (or what is also called “high sensitivity” testing) refers to the testing and analysis of very small amounts of DNA, often involving special techniques to increase the sensitivity of the test. LCN results are sometimes characterised by stochastic or random effects that could radically affect their interpretation.⁷

The complexities and potential pitfalls regarding LCN tests discussed in this article make it imperative for defence counsel always at the outset of criminal proceedings to ascertain whether DNA evidence proffered was, in fact, obtained by the LCN DNA testing method.

The very nature of DNA transfer, as briefly noted below, is inherently complex. The lack of availability of substantial DNA samples resulting in trace evidence further complicates establishing DNA profiles. Gill⁸ notes the following limitations with regard to trace evidence, that should always be borne in mind when dealing with very small samples of DNA:

- Although a DNA profile has been obtained, it is possible neither to identify the type of cells from which the profile originated, nor to state *when* the cells were deposited.

2 Butler 2015.

3 Sowmyya “Touch DNA: An investigative tool in Forensic Science” 25993-25994, <https://www.journalcra.com/sites/default/files/issue-pdf/12921.pdf> (accessed on 21 October 2020).

4 Lebrecht “The controversy of using low copy number DNA analysis in forensic science”, <http://dnapolicyinitiative.org/the-controversy-of-using-low-copy-number-dna-analysis-in-forensic-science/> (accessed on 19 May 2020).

5 Press “DNA mixtures: A forensic science explainer”, <https://www.nist.gov/featured-stories/dna-mixtures-forensic-science-explainer> (accessed on 7 January 2021).

6 Gill 2014.

7 *US v Wilbern* United States District Court Western District of New York 17-CR-6017 CJS 09-06-2019.

8 Gill 2014:2.

- It is not possible to make any conclusion about transfer and persistence of DNA in this case.
- Because the DNA test is very sensitive, it is not unexpected to find mixtures. If the potential origins of DNA profiles cannot be identified, it does not necessarily follow that they are relevant to this case, since transfer of cells can occur as a result of casual contact.

1.2 Delineating concepts

DNA material described as low copy⁹ or low template material¹⁰ is often referred to by the terms “trace DNA” or “touch DNA”.¹¹ Trace DNA samples, which have been defined by Van Oorschot *et al.* as “any sample which may fall below recommended thresholds at any stage of the analysis”, from sample detection through to profile interpretation, cannot be defined by a precise picogram amount.¹² Gill adapted Van Oorschot’s definition of trace DNA and describes this phenomenon as “any sample where there is uncertainty that it may be associated with the crime event itself – so that it is possible that the transfer may have occurred before the crime event (innocent transfer) or after the crime event (investigator mediated).”¹³ The terms “low template (LT DNA)”, “low copy (LCN)” or “trace/touch DNA” refer to deposited DNA that weighs less than 200 picograms.¹⁴ Conventional STR kits normally used for DNA profiling are designed to be applicable in circumstances where at least 200 picograms of DNA are available for analysis.¹⁵ In South Africa, between 100 picograms and 1000 picograms (one nanogram) are normally processed for the purpose of DNA profiling.¹⁶ Although only samples that are above 100 picograms and below 200 picograms are tested in South Africa by the LCN method, the problems that pertain to LCN DNA testing are also applicable to the South African situation.¹⁷

Low copy number DNA (LCN DNA) is sometimes referred to as low template (LT DNA). The term LCN was coined by the Forensic Science

9 Gill *et al.* 2000:17-40; Gill P 2001:229-232.

10 Caddy *et al.* 2008.

11 Van Oorschot *et al.* 2010; Sessa *et al.* 2019.

12 Van Oorschot *et al.* 2010.

13 Gill 2014:2; Gill 2016:10.

14 Word “What is LCN? – Definitions and challenges”, <https://worldwide.promega.com/resources/profiles-in-dna/2010/what-is-lcn-definitions-and-challenges/> (accessed on 24 May 2020).

15 Budowle “DNA low copy number typing still lacks robustness”, <https://worldwide.promega.com/resources/profiles-in-dna/low-copy-number-typing-still-lacks-robustness-and-reliability/#referenceList> (accessed on 4 January 2021).

16 Lieutenant Colonel Sharlene Otto, Sub-section commander. E-mail correspondence: DNA Reporting Biology SAPS FSL (18 May 2020).

17 Lieutenant Colonel Sharlene Otto, Sub-section commander. E-mail correspondence: DNA Reporting Biology SAPS FSL (18 May 2020).

Service (FSS)¹⁸ in England and Wales to refer to the method, in terms of which the PCR cycle number is increased for the purpose of low template (LT DNA) analysis.¹⁹ Courts in the United States of America (USA)²⁰ and in England and Wales²¹ have ruled on a number of aspects pertaining to the testing of LCN DNA.²² Although the term LCN DNA analysis is often restricted to the process used in England and Wales, LCN DNA and LT DNA utilise similar techniques.²³ Jamieson²⁴ points out that, whereas the DNA profiling kit normally used recommends an amplification of 28 times, the LCN method attempts to improve analysis sensitivity by increasing amplification to 34 times.²⁵

According to Jamieson,²⁶ the LCN has two components namely:

the multiplication of the small numbers of DNA molecules in the sample to produce enough to be seen by the analytical equipment, (...) the interpretation of the results produced by the physical processes.²⁷

Evans and Hadi²⁸ emphasise that the method of interpretation is significant, since “the lower the amount of DNA present in a sample, the greater the chance that it may not be associated with a crime-event”.²⁹ Proper interpretation, as

18 Before its closure in March 2012, the Forensic Science Service (FSS), a company owned by the government of the UK, furnished the police forces of England and Wales as well as some other countries with forensic science services.

19 Grisedale 2014:14.

20 *People of California v Hector Espino* NA076620 (Los Angeles County Superior Court March 18, 2009); *United States v Davis* 602 F. Supp 2d 658 (D Md 2009); *United States v Williams* 2009 WL 1704986 (C.D Cal. 2009); *People v Megnath* 898 N.Y.S.2d 408 (Sup. Ct. 2010); Ankney “South Carolina Supreme Court overturns murder conviction where state presented improper testimony regarding trace DNA evidence”, <https://www.criminallegalnews.org/news/2020/jul/15/south-carolina-supreme-court-overturns-murder-conviction-where-state-presented-improper-testimony-regarding-trace-dna-evidence/> (accessed on 20 October 2020). See also Word “What is LCN? – Definitions and challenges”, <https://worldwide.promega.com/resources/profiles-in-dna/2010/what-is-lcn-definitions-and-challenges/> (accessed on 24 May 2020).

21 *R v Hoey* 2007 NICC 49; *R v Reed and Reed*; *R v Garmson* [2010] 1 Cr App R 23; [2009] EWCA Crim 2698. See also Word “What is LCN? – Definitions and challenges”, <https://worldwide.promega.com/resources/profiles-in-dna/2010/what-is-lcn-definitions-and-challenges/> (accessed on 24 May 2020).

22 *R v Hoey* 2007 NICC 49; *R v Reed and Reed*; *R v Garmson* [2010] 1 Cr App R 23; [2009] EWCA Crim 2698.

23 The Forensic Institute “Low copy number or low template DNA analysis”, <http://www.theforensicinstitute.com/news-articles/research/dna/low-template-or-low-copy-number> (accessed on 19 May 2020); Burns “Low copy number DNA on trial”, <https://www.newlawjournal.co.uk/content/low-copy-number-dna-trial> (accessed on 7 June 2020).

24 Jamieson 2011:163.

25 Jamieson 2011:163.

26 Jamieson 2011:164.

27 Jamieson 2011:164.

28 Evans & Hadi 2018:1.

29 Gill *et al.* 2015:104.

informed by the prescribed guidelines and protocols pertaining to interpretation, coupled with the fact that two analysts work on the same sample separately, could assist in avoiding associated risks such as biased results that could lead to wrongful convictions and the danger of miscarriages of justice.³⁰

2. MODES OF DNA DEPOSITION

When evaluating trace DNA evidence and its reliability, it is important to consider the different possible modes of deposition.³¹ DNA can be deposited by means of three different modes of transfer, namely primary, secondary, or tertiary transfer.

DNA can be directly deposited on a surface as a result of contact by an individual or by bodily excretion such as blood, vomit, excreta, and semen, resulting in a primary transfer.³² Evans and Hadi point out that DNA “can be transferred up to 155cm during speech and a static speaking individual may contaminate their immediate environment in as little as thirty seconds”.³³

When DNA is transferred from one person to another person or an object, via another person or object, the process is referred to as secondary transfer.

Rutty³⁴ describes a third mode of DNA transfer, namely tertiary transfer. In such an instance, a third party is involved when DNA is transferred from a person to another person or object having passed through two or more intermediaries.³⁵

When DNA is transferred to a surface by an intermediary such as in the case of secondary and tertiary transfer, there is always the possibility of a mixed DNA sample being found.³⁶ It is, therefore, of vital importance clearly to distinguish between a sample deposited by a suspect and any background DNA that might be present.³⁷

30 Page 2014:57-75.

31 Evans & Hadi 2018:1.

32 Evans & Hadi 2018:1-2.

33 Evans & Hadi 2018:1.

34 Rutty 2002:171; Evans & Hadi 2018:2.

35 Rutty 2002:171; Evans & Hadi 2018:2.

36 See Australian case of *Fitzgerald v R* 2014 HCA 28. In the initial trial of Fitzgerald, the appellant, the prosecution contended that Fitzgerald was a member of a group of armed persons who raided a house with the intent to cause harm to the inhabitants of the house. In the absence of any evidence that Fitzgerald caused harm to the deceased or to the other victim, the prosecution relied on DNA evidence found on a didgeridoo left at the crime scene. The prosecution assumed that Fitzgerald's DNA was deposited on the didgeridoo during the attack. The defence contended that there were other ways in which Fitzgerald's DNA could have been transferred to the didgeridoo. One possibility was that Fitzgerald could have shaken hands with a culprit involved in the attack and consequently his DNA could have reached the didgeridoo by means secondary transfer.

37 Evans & Hadi 2018:1-5.

3. CHALLENGES TO THE INTERPRETATION OF TRACE DNA

The amounts of DNA available for analysis could be minute or fragmented and consequently negatively impact on the quality of the sample being examined.³⁸ As a consequence of the above, there could be a failure to produce a profile, or the quality of the profile could be reduced.³⁹ Careful interpretation of the results is crucial, since analysis of low level DNA samples could be confronted by a number of problems such as allelic⁴⁰ drop-out, allelic drop-in, elevated stutter peaks, severe peak imbalances, contamination and DNA mixtures of two or more persons.⁴¹ These concepts are explained below.

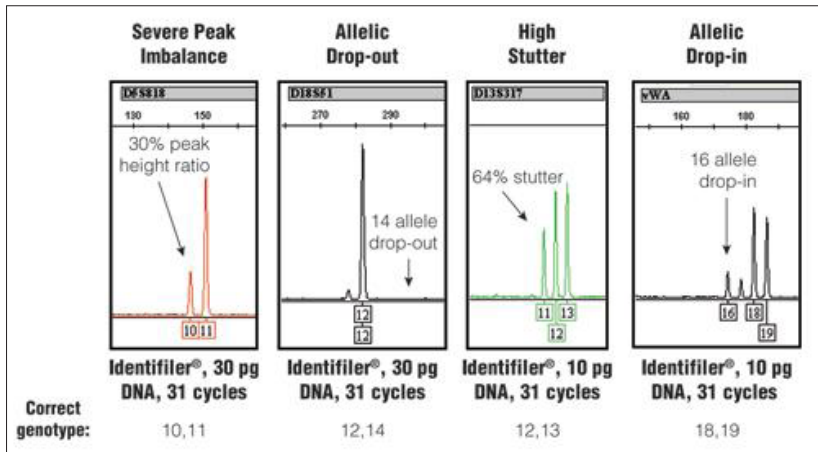


Figure 1: “Stochastic effects that randomly occur when PCR amplifying low amounts of DNA using an increased number of PCR cycles.”⁴²

3.1 Allele drop-out

Drop-out, which is an extreme form of peak height imbalance, occurs when a piece of DNA is not detected by the testing process, because the quantity of DNA being tested is so small. Thus, pieces of DNA that belong to the DNA profile of a contributor to a sample are literally missing.⁴³ When, at heterozygote locus, one pair of alleles are not amplified to a level that is detectable, this results in what is referred to as allele drop-out.⁴⁴ This means that there is a

38 Gill *et al.* 2009:250-267; Forster *et al.* 2008:318-328.

39 Gill 2000:17-40; Caragine *et al.* 2008:318-328.

40 An allele is one of the alternative forms of a gene. “Allelic” is the adjective of allele.

41 Butler & Hill 2010.

42 Butler & Hill 2010.

43 *US v Wilbern* United States District Court Western District of New York 17-CR-6017 CJS 09-06-2019.

44 Timken *et al.* “Stochastic sampling effects in STR typing: Implications for analysis and interpretation”, [https://www.fsigenetics.com/article/S1872-4973\(14\)00073-8/pdf#%20](https://www.fsigenetics.com/article/S1872-4973(14)00073-8/pdf#%20) (accessed on 27 December 2020).

possibility that some alleles of individuals in a contributing community might not be observed. In samples containing minute amounts of DNA, stochastic effects could result in a situation where the PCR reaction does not sufficiently portray the template of some of the alleles.⁴⁵ Technical imperfections in the methods used for analysis could be a reason why an amplified allele could not meet the necessary signal-to-noise threshold.⁴⁶ This may lead to incomplete profiles and inaccurate interpretation of the DNA profiles and could inevitably result in a wrongful conviction. With LCN DNA testing, there is an increased chance of allelic “drop-out”.

3.2 Allele drop-in (stutter false alleles)

This term is used to describe one or two “foreign” alleles per DNA profile.⁴⁷ Allele drop-in occurs when a false allele is observed and is not reproducible.⁴⁸ Additional alleles are present in a profile originating from random fragmented sources and are regarded as independent events (no more than two events per profile allowed).⁴⁹ In instances where the process is repeated several times and where the obtained results are not identical, it could be assumed that drop-in occurred.⁵⁰ Drop-in events, as opposed to contamination, occur independently of markers.⁵¹ Drop-in occurs when the testing detects pieces of DNA that are not part of the crime scene sample but become part of the test results. This is greatly exacerbated with increased PCR cycles that increase the sensitivity of the test, thus increasing the picking up of contaminants.⁵²

3.3 Elevated stutter peaks

Elevated stutter marks are “non-allelic peaks in a DNA profile that occur due to over or under replication during the PCR process”.⁵³ LCN (31-cycle) PCR testing often causes an increase in the height of stutter artefacts. “Stutter” is

45 Timken *et al.* “Stochastic sampling effects in STR typing: Implications for analysis and interpretation”, [https://www.fsigenetics.com/article/S1872-4973\(14\)00073-8/pdf#%20](https://www.fsigenetics.com/article/S1872-4973(14)00073-8/pdf#%20) (accessed on 27 December 2020).

46 Evans & Hadi 2018:3; Gill *et al.* 2015:113.

47 Forensic Science Regulator Guidance “The interpretation of DNA evidence (including low-template DNA)” FSR-G-202 Issue 2 at 15, https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/918731/FSR-G-202_Interpretation_of_DNA_evidence_Issue_2_Final__002_.pdf (accessed on 30 December 2020). See also Gill *et al.* 2000:17-40.

48 Butler 2005:167-170.

49 Forensic Science Regulator Guidance “The interpretation of DNA evidence (including low-template DNA)” FSR-G-202 Issue 2, https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/918731/FSR-G-202_Interpretation_of_DNA_evidence_Issue_2_Final__002_.pdf (accessed on 30 December 2020).

50 Wulff 2006:2.

51 Wulff 2006:2.

52 *US v Wilbern* United States District Court Western District of New York 17-CR-6017 CJS 09-06-2019 at 15.

53 *US v Wilbern* United States District Court Western District of New York 17-CR-6017 CJS 09-06-2019 at 16.

the name for the product of a “mistake” in the PCR process: that is, the DNA strand being copied during PCR slips and bulges, and therefore appears to be a DNA peak on a printed electropherogram to be interpreted by an analyst. Stutter is an artefact and is not a real indication of the presence of a gene, although it looks like a piece of DNA (a peak on an electropherogram).⁵⁴ Stutter is a well-known phenomenon even in conventional DNA testing and is usually recognised in routine testing, because it is only a certain percentage of the height of the real piece of DNA next to it. Stutter phenomena, however, are problematic with LCN testing, because the height of stutter increases proportionally to a true allele (real piece of DNA) and is, therefore, difficult to identify as an artefact as opposed to a real allele. This increased challenge complicates the interpretation of an electropherogram, making results potentially less reliable.⁵⁵ *S v SB*⁵⁶ illustrates the dangers of dropping in/out in artefact interpretation in DNA profiles, although LCN typing was not used in that case.

3.4 Severe peak imbalances

Severe peak imbalances occur “when two alleles that should be matching, or homozygous, appear to have different values”.⁵⁷ Peak height imbalances are calculated in heterozygous loci (containing two alleles).⁵⁸ When the difference in the height of the peaks is more than 30 per cent, it is normally considered to be a peak height imbalance.⁵⁹ An imbalance of peaks would normally indicate a mixture. Peaks from a single person sample should be more or less the same in height.⁶⁰ With LCN testing, peak height imbalance is increased, which can result in variations of the heights of peaks (alleles) belonging to one contributor and lead to the misrepresentation of the evidence.

3.5 Contamination

The *DNA Forensic interpretation: A primer for courts of the Royal Society*⁶¹ states that:

Contamination in the context of DNA analysis can be defined as the introduction of extraneous DNA (or biological material containing DNA) to a sample. The DNA profiling process is extremely sensitive and constant vigilance is required to ensure that contamination does

54 *US v Wilbern* United States District Court Western District of New York 17-CR-6017 CJS 09-06-2019 at 16.

55 *US v Wilbern* United States District Court Western District of New York 17-CR-6017 CJS 09-06-2019 at 16.

56 *S v SB* 2014 (1) SACR 66 (SCA).

57 Gill *et al.* 2015:103. Caddy *et al.* 2008:par. 9.1; Craig 2017:186.

58 Forensic Bioinformatics “Possible issues with DNA evidence”, <http://www.bioforensics.com/dna-testing-issues/> (accessed on 26 December 2020).

59 Taylor *et al.* 2016:126-133. See also *US v Wilbern* United States District Court Western District of New York 17-CR-6017 CJS 09-06-2019 at 16.

60 Forensic Bioinformatics “Possible issues with DNA evidence”, <http://www.bioforensics.com/dna-testing-issues/> (accessed on 26 December 2020).

61 The Royal Society *DNA Forensic Interpretation: A primer for courts* (2017).

not affect the results. Because of this sensitivity, contaminating DNA may still be observed even with careful precautions, and will routinely be monitored in laboratories. The forensic scientist must use all the information available to them to assess whether a contamination event, if it occurs, has had an impact on the results in a specific case.⁶²

In dealing with trace DNA, the possibility of contamination should always be considered as background DNA, DNA unrelated to the crime, or even contamination that occurred after the crime was committed (for example, laboratory contamination) could have contributed to a mixed sample.⁶³ Because of this sensitivity, the LCN testing process requires purpose-built and strictly managed laboratories and facilities, with special protocols.⁶⁴ The processing of trace DNA could be difficult as a result of the inherent complexities of low-level DNA and/or as a result of contamination stemming from the way in which the sample was handled or from shortcomings in laboratory processes.⁶⁵ PCR amplification often results in a situation where analysts observe background DNA contamination caused by the fact that several people other than the suspected criminals handled the sample and left observable DNA.⁶⁶ In such circumstances, it becomes difficult to identify and distinguish the real DNA profile from the DNA of the contaminants. The chain of evidence is important to show that no tampering or substantial alterations of the evidence occurred during the collection, sealing, safekeeping, sending and receipt by the forensic laboratory for analysis.⁶⁷

3.6 Mixed profiles

Inherent stochastic effects could make it extremely difficult successfully to analyse and resolve mixed samples where DNA from more than one person is present.⁶⁸ According to the Forensic Institute:

the reliability of the LCN method below the stochastic threshold has not been demonstrated with sufficient numbers of samples and with samples which represent those likely to be discovered in crime stains. The limited extant data must be made available to enable the scientific community to conduct a meaningful assessment of the inferences that can be made from it.⁶⁹

In instances where significant stochastic effects are not present, it is not likely that the LCN DNA technique would give rise to contention.⁷⁰ However, in situations where there are stochastic effects, the interpretation of the LCN

62 The Royal Society *DNA Forensic Interpretation: A primer for courts* (2017):13.

63 Van Oorschot *et al.* 2010:12.

64 *R v Cassidy* 2016 NTSC 1.

65 Evans & Hadi 2018:1.

66 McBride 2012:13.

67 Meintjes-Van der Walt 2010:373.

68 Grisedale 2014:2.

69 The Forensic Institute "Low copy number or low template DNA analysis", <http://www.theforensicinstitute.com/newsarticles/research/dna/lowtemplateorlowcopynumber> (accessed on 19 May 2020).

70 Jamieson 2011:165.

analysis will be open to challenge. LCN DNA typing is further complicated when the sample contains DNA from persons belonging to the same gender group.⁷¹ It might also be difficult to decide whether a sample is indeed a mixture and, if it is a mixture, individualising the DNA from the different contributors could confront analysts with a real challenge in determining the origin of alleles.⁷²

Where trace DNA samples contain DNA from three or more contributors, interpretation uncertainties and the potential for errors increase.⁷³

Stochastic effects are especially problematic in LCN DNA mixtures, which are inherently challenging to interpret.

4. DETERMINING THE WEIGHT OF THE LCN DNA ANALYSIS

4.1 Case law and reports

To date, there have not been any reported cases in South Africa where LCN DNA has been used. Reliability can be a factor to determine the weight that should be attached to results from LCN DNA testing.⁷⁴ While in other Anglo-American systems, reliability is a factor that plays a major role in the admissibility of expert evidence, in South Africa recently the court in *Twine v Naidoo* also held that, for expert evidence to be admissible, such evidence should not only be relevant but also reliable.⁷⁵ In any event, the weight of LCN DNA evidence will depend on the reliability of the LCN testing results.⁷⁶ According to McBride:

The trouble is that it is difficult to tell which if any peaks are missing or falsely present, and even if the sample was to be run through the machine a number of times, the same result may not necessarily be produced each time – which can clearly lead to unreliable test results. This is a major problem with LCN, because the cornerstone of good scientific method requires that results are *reproducible*. What this means for a criminal case is that it may produce a result that is a whole or partial profile that does or does not match the accused, but if the results cannot be produced reliably – how reliable is it all?⁷⁷

71 Marshall 2014:22.

72 Budowle *et al.* “Low copy number – Consideration and caution, genetic identity conference proceedings. Twelfth International Symposium on Human Identification”, <http://www.promega.com/geneticidproc/ussymp12proc/contents/budowle.pdf> (accessed on 25 September 2020). The author intends to explore DNA mixtures more fully in an upcoming full-length article.

73 Butler 2015:5.

74 Meintjes-Van der Walt 2001:206-207.

75 *Twine v Naidoo* (38940/14) [2017] ZAGPJHC 288; [2018] 1 All SA 297 (GJ) (16 October 2017).

76 See also Meintjes-Van der Walt 2003:91-106, where the author suggests factors that can impact on the weight of the expert evidence, in cases where the only admissibility requirement is relevance.

77 McBride 2012:10.

The reliability of the results of trace DNA is influenced by the stochastic effects discussed above.⁷⁸

The LCN DNA testing technique's reliability was criticised by the Crown Court of Northern Ireland in *R v Hoey*⁷⁹ (the so-called "Omagh bombing trial"). Hoey was charged with a number of offences arising from thirteen incidents, including the Omagh car bomb and mortar attacks on army bases in Northern Ireland.⁸⁰ The prosecution's case consisted, *inter alia*, of the LCN DNA analysis that was done to provide evidence of contact between the accused and phenomena pertinent to the bombs and mortar attacks.⁸¹ After the events of 1998/1999, a number of items relevant to this case were recovered and subjected to LCN DNA examination. However, the investigators did not exercise the required precautions regarding the recovery, storage, and transmission of DNA material necessary for a reliable LCN DNA examination.⁸²

Regarding the DNA evidence pertaining to this case, Weir J stated that the defence team:

uncovered very many unsatisfactory matters. I do not propose to list all of those here but rather to give examples to exemplify the types of problems uncovered. It is highly important in this connection to bear in mind that, given the tiny amount of material needed to give a result using the LCN DNA technique, everyone agreed that especially stringent measures must be taken to avoid the contamination of samples. Dr Griffin indicated that the protective measures in the laboratory have been enhanced since the advent of LCN and the awareness of the need for the wearing of masks and hair covering to prevent the transfer of DNA from the examiner onto the item. The Defence submit, correctly in my judgment, that it is for the prosecution to establish the integrity and freedom from possible contamination of each item throughout the entirety of the period between seizure and any examination relied upon. They contend, and I accept the contention, that the court must be satisfied by the prosecution witnesses and supporting documents that all dealings with each relevant exhibit have been satisfactorily accounted for from the moment of its seizure until the moment when any evidential sample relied upon by the prosecution is taken from it and that by a method and in conditions that are shown to have been reliable.⁸³

Weir J further explained:

This means that each person who has dealt with the item in the intervening period must be ascertainable and be able to demonstrate

78 *R v C* [2010] EWCA Crim 2578:par. 27; Jamieson 2011:165.

79 *R v Hoey* [2007] NICC 49 (20 December 2007); Jamieson 2011:161.

80 *R v Hoey*:paras. 30, 45-46.

81 *R v Hoey*:paras. 30, 45-46; Burns "Low copy number DNA on trial", [https://www.newLawjournal.rnal.co.uk/content/low-copy-number-dna-trial](https://www.newlawjournal.rnal.co.uk/content/low-copy-number-dna-trial) (accessed on 7 June 2020).

82 *R v Hoey*:par. 30; Burns "Low copy number DNA on trial", <https://www.newLawjournal.co.uk/content/low-copy-number-dna-trial> (accessed on 7 June 2020).

83 *R v Hoey*:par. 46.

by reference to some proper system of bagging, labelling, and recording that the item has been preserved at every stage free from the suspicion of interference or contamination.⁸⁴

Weir J continued:

For this purpose [the prosecution] must be able to demonstrate how and when and under what conditions and with what object and by what means and in whose presence he or she examined the item.⁸⁵

Weir J criticised the way in which the DNA evidence adduced at the trial was handled and thus rejected the evidence, since he had significant concerns about the validity of the LCN DNA technique.⁸⁶ Following this critical judgment, the use of LCN DNA in England and Wales in criminal investigations was suspended from December 2007 until January 2008.⁸⁷ The purpose of the suspension was to allow for a review of the use of LCN DNA evidence in trials and to ensure that the implications of the “Omagh bombing trial” regarding the LCN profiling were addressed before the technique was used in future proceedings.⁸⁸ In 2008, the English Crown Prosecution Service (CPS) stated that it had “not seen anything to suggest that any current problems exist with LCN” and that it should remain available as potentially admissible evidence.⁸⁹ The CPS press release further states that:

[a]t present, there is no reason to believe that there is any inherent unreliability in the LCN DNA analysis process provided that it is carried out according to the prescribed processes, and that the results are properly interpreted. In its work so far, the review has found nothing that would indicate any serious flaw in the scientific principles.⁹⁰

As recommended by the CPS, a review, known as the Caddy Review, was conducted in relation to the LCN DNA profiling techniques, the validity of the techniques, the interpretation of results, and the creation of a minimum

84 *R v Hoey*:par. 46.

85 *R v Hoey*:par. 46.

86 *R v Hoey*:paras. 59-61.

87 Burns “Low copy number DNA on trial”, [https://www.newLawjournal.co.uk/content/low-copy-number-dna-trial](https://www.newlawjournal.co.uk/content/low-copy-number-dna-trial) (accessed on 7 June 2020).

88 Burns “Low copy number DNA on trial”, <https://www.newLawjournal.co.uk/content/low-copy-number-dna-trial> (accessed on 7 June 2020).

89 CPS Press Release “Review of the use of low copy number DNA analysis in current cases: CPS statement”, <https://www.newLawjournal.co.uk/content/low-copy-number-dna-trial> (accessed on 7 June 2020); CPS Press Release “Review of the use of low copy number DNA analysis in current cases: CPS statement”, <https://insidetime.org/download>, https://insidetime.org/download/miscarriage_of_justice/dna/DNA_Low_Copy_No_CPS_Statement_2008.pdf (accessed on 8 June 2020). See also Burns “Low copy number DNA on trial”, <https://www.newLawjournal.co.uk/content/low-copy-number-dna-trial> (accessed on 7 June 2020).

90 CPS Press Release “Review of the use of low copy number DNA analysis in current cases: CPS statement”, https://insidetime.org/download/miscarriage_of_justice/dna/DNA_Low_Copy_No_CPS_Statement_2008.pdf (accessed on 8 June 2020).

technical standard for LCN DNA analysis.⁹¹ According to the Caddy Review, “a key question is whether or not the process(es) involved in LCN DNA analysis have been adequately validated and whether such a validation is accepted by the international forensic science community.”⁹²

In the *Hoey* case, Weir J commented on the LCN DNA validation process:

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 and monitored.⁹³

The Caddy Reviewers agreed with Weir J’s validation comments.⁹⁴ In view of Weir J’s questions on the validity and reliability of LCN DNA technique, the authors of the Caddy Review were of the 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.⁹⁵

The Caddy Report further stated that:

[r]eservations 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 LT DNA analysis (including LCN) providing all the appropriate conditions are met.⁹⁶

The Caddy reviewers agreed that the LCN or LT DNA technique is fit for purpose and were of the view that the forensic science community should implement it internationally.⁹⁷ They also emphasised the fact that any LT DNA profile should always be reported to a jury with specific caveats.⁹⁸ The caveats referred to above, are the following:

- That the nature of the original starting material is unknown;

91 Caddy *et al.* 2008:par. 1.1.

92 Caddy *et al.* 2008:par. 3.13.

93 *R v Hoey*:par. 62.

94 Caddy *et al.* 2008:par. 3.14.

95 Caddy *et al.* 2008:par. 7.2.

96 Caddy *et al.* 2008:par. 7.3.

97 Caddy *et al.* 2008:par. 7.3.

98 Caddy *et al.* 2008:par. 7.4.

- That the time at which the DNA was transferred cannot be inferred;
- That the opportunity for secondary transfer is increased in comparison to standard DNA profiling;⁹⁹
- 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, and
- 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.¹⁰⁰

In 2009, the admissibility and reliability of LCN DNA analysis in criminal proceedings were disputed in England and Wales in the Court of Appeal in *R v Reed and Reed; R v Garmson* (hereafter, the *Reed and Reed* case).¹⁰¹ The court observed that “there is no agreement among scientists as to the precise line where the stochastic threshold should be drawn, but it is between 100 and 200 picograms¹⁰² of DNA material”.¹⁰³ If the analysis does not suffer from stochastic effects, the result from the LCN DNA would be reliable and admissible.¹⁰⁴ The court concluded that:

a challenge to the validity of the method of analysing Low Template DNA by the LCN process should no longer be permitted at trials where the quantity of DNA analysed is above the stochastic threshold of 100-200 picograms in the absence of new scientific evidence.¹⁰⁵

The court in the *Reed and Reed* case thus set a minimum standard for what is required if LCN DNA evidence is admitted in a trial. The court was of the view that the LCN DNA technique could be used to obtain profiles that are reliable when interpreted in instances where the quantity of the DNA available for analysis is above the stochastic threshold.¹⁰⁶

In the USA, DNA evidence tested with the LCN method was first accepted in the state of New York in 2010¹⁰⁷ in *People v Megnath* (hereafter, the *Megnath* case).¹⁰⁸ In the *Megnath* case, the accused was charged with murder. Small amounts of DNA-containing material, which linked him to the murder, were found in the accused’s automobile.¹⁰⁹ The LCN analysis was used to test

99 *Caddy et al.* 2008: para 7.4.

100 *Caddy et al.* 2008:par. 7.5.

101 *R v Reed and Reed; R v Garmson* [2010] 1 Cr App R 23; [2009] EWCA Crim 2698.

102 Above 200 picograms ordinary PCR will take place, in other words, 28 times. See Jamieson 2011:164.

103 *R v Reed and Reed; R v Garmson*:par. 74. See Jamieson 2011:162.

104 Jamieson 2011:162.

105 *R v Reed and Reed; R v Garmson*:par. 74.

106 *R v Reed and Reed; R v Garmson*:par. 74; Jamieson 2011:162.

107 Craig 2017:188. See also *People v Megnath* 898 N.Y.S.2d 408 (Sup. Ct. 2010) at 412.

108 *People v Megnath* 898 N.Y.S.2d 408 (Sup. Ct. 2010).

109 *People v Megnath* 898 N.Y.S.2d 408 (Sup. Ct. 2010) at 407.

the DNA samples.¹¹⁰ The accused questioned the reliability and acceptance of LCN in the relevant scientific community. The court noted that the LCN method had been used across the world for ten years and is generally accepted as reliable.¹¹¹ The court consequently found the LCNDNA evidence to be admissible at trial.¹¹²

In 2013, the court in *United States v McCluskey*¹¹³ in New Mexico did not include the results of LCN DNA testing, as it found the testing to be unreliable. PCR was used to amplify the samples to create a DNA profile and, as a consequence, the profile exhibited stochastic effects such as allele drop-out.¹¹⁴ The court ruled that the *Megnath* decision was not relevant in this instance, reasoning that the stochastic effects of LCN DNA were significant in this particular case.¹¹⁵ After considering the effects or risks of the LCN method, the court did not find LCN evidence in this particular case to be reliable or admissible.¹¹⁶

In *Wallace v R* (hereafter, the *Wallace* case),¹¹⁷ the New Zealand Court of Appeal, in 2010, dismissed the appeal of a man found guilty of the murder of a backpacker. The murder weapon, a metal bar, linking the appellant to the crime, contained traces of DNA.¹¹⁸ In this particular case, the use of LCN DNA processing produced a low template profile that matched the DNA profile of the victim.¹¹⁹ Hammond J, in the *Wallace* case, held that a blanket attack on LCN DNA processing was no longer tenable, as LT DNA profiles were accepted as evidence in trial proceedings in other jurisdictions such as the USA, the United Kingdom (UK), and Australia.¹²⁰ As such, any challenge to the admissibility or reliability of the LT DNA must be based on the facts of a particular case.¹²¹

The critical question in the *Wallace* case was whether the low template profile was sufficiently reliable.¹²² It is important to note that the integrity of the profile had been attested to at the trial by qualified experts, and the defence did not challenge the DNA evidence.¹²³ Furthermore, the prosecution had built a strong case based on circumstantial and identification evidence.¹²⁴

110 *People v Megnath* 898 N.Y.S.2d 408 (Sup. Ct. 2010) at 409.

111 *People v Megnath* 898 N.Y.S.2d 408 (Sup. Ct. 2010) at 410-414.

112 *People v Megnath* 898 N.Y.S.2d 408 (Sup. Ct. 2010) at 413-414.

113 *United States v McCluskey* 954 F.Supp. 2d 1224 (D.N.M 2013) at 1288.

114 *United States v McCluskey* 954 F.Supp. 2d 1224 (D.N.M 2013) at 1276-1278.

115 *United States v McCluskey* 954 F.Supp. 2d 1224 (D.N.M 2013) at 1279-1280.

116 *United States v McCluskey* 954 F.Supp. 2d 1224 (D.N.M 2013) at 1280-1281, 1288. See also Craig 2017:193.

117 *Wallace v R* [2010] NZCA 46. See also Pattenden 2010:262.

118 *Wallace v The Queen* [2010] NZCA 46:par. 27.

119 *Wallace v The Queen*:par. 29.

120 *Wallace v The Queen*:par. 99.

121 *Wallace v The Queen*:par. 100; Pattenden 2010:262.

122 *Wallace v The Queen*:par. 112.

123 *Wallace v The Queen*:par. 112; Pattenden 2010:262.

124 *Wallace v The Queen*:par. 118.

In Italy, the LCN DNA analysis was also used in the case of Amanda Knox and Raffaele Sollecito.¹²⁵ Knox was convicted of the murder of her housemate, Meredith Kerchner, in 2007, after a low copy sample of her DNA was found on the handle of a knife, kept in her boyfriend's kitchen. The victim's DNA was also found on the knife, but the kitchen knife did not match any wounds on the body of the deceased and tested negative for blood. DNA from Knox was on the handle of the knife, which she previously had used for preparing food. On one swab from the blade, a minuscule trace of DNA was detected, only once during several analyses. It contained DNA that was consistent with that of the victim. Despite several attempts, this finding was not repeated. The question arose as to whether a single result under such circumstances could be regarded as reliable.¹²⁶ Sollecito, Knox's boyfriend, was also convicted after traces of his DNA were found on the victim's bra clasp. The two were later exonerated following a forensic report, which revealed that the DNA evidence used to convict the two was unreliable and possibly contaminated.¹²⁷ It should be noted that this forensic report does not suggest that LCN DNA analysis is not reliable, but rather that defence counsel should be acutely aware of the potential for error and contamination.

Although the Forensic Science Laboratory (FSL) in South Africa performs analyses from trace DNA of between 100 picograms and 200 picograms,¹²⁸ the LCN method has to date not been discussed in any reported cases. Nevertheless, the conclusions that follow could, in future, be of value to legal practitioners.

5. CONCLUSION

Weir J pointed out in the *Hoey* case that "justice 'according to law' demands proper evidence ... evidence which is so convincing in truth and manifestly reliable that it reaches the standard of proof beyond reasonable doubt",¹²⁹ but the evidence, which was primarily based on LCN DNA evidence, was found to fail the required standard of proof. This led to the suspension of the LCN DNA technique, resulting in a review of the technique by the CPS. The subsequent Caddy Review concluded that there is no reason to assume that there is any inherent unreliability in LCN DNA testing. If LCN DNA testing is conducted in

125 See Matthews "Injustice in Perugia", <http://www.injusticeinperugia.org/CaseSummary.html> (accessed on 8 May 2021); Gill 2016:23.

126 Gill 2016:23.

127 Marasca-Bruno Supreme Court of Cassation motivation report", <http://www.amandaknoxcase.com/wp-content/uploads/2015/09/> (accessed on 15 July 2020); Gill 2016:9-18.

128 Lieutenant Colonel Sharlene Otto, Sub-section commander. E-mail correspondence: DNA Reporting Biology SAPS FSL (18 May 2020).

129 *R v Hoey*:par. 65. See also *R v Steenson* [1986] NIJB 17 at 36.

accordance with the prescribed processes and if the results are interpreted accurately, then the LCN technique in a particular case will be reliable.¹³⁰

There are, however, other issues that should be considered. While LCN DNA evidence may improve with time as laboratory research advances, it is important to be cautious when evaluating DNA profile results obtained in this manner. Mayne¹³¹ recommended that the following factors should be borne in mind:

1. Identify which DNA typing PCR kit was used by the laboratory.
2. Review the PCR method to see the DNA concentration used for the sample, and the number of PCR cycles.
3. Do the laboratory procedures include any modifications to the standard operating protocols to increase the sensitivity of detection?
4. Identify if the laboratory has performed validation studies for PCR conditions outside the standard operating protocols.
5. Is the DNA typing PCR kit subjected to quality control at these conditions?
6. Were the alleles interpreted using standard laboratory protocols or laboratory protocols for modified PCR conditions?
7. Review negative controls for spurious bands.
8. Does the reporting scientist understand the limitations in reporting and interpretation of LCN typing? Were the results validated by reproducibility – multiple amplifications of the same sample? Good laboratory practice would include repeating the sample from the same area, and this is a recommendation of Gill in the use of LCN. If this is not possible due to a small amount of sample, then is it reasonable to rely on such evidence?¹³²

While LCN DNA technology undoubtedly has significant potential applications in crime investigation, Sowmya points out that the following limitations of LCN DNA should be considered:

1. Contamination of touch DNA caused by the contaminants present on the target surface. Trace DNA mixtures interpretation is difficult.

130 CPS Press Release “Review of the use of low copy number DNA analysis in current cases”: CPS statement, https://insidetime.org/download/miscarriage_of_justice/dna/DNA_Low_Copy_No_CPS_Statement_2008.pdf (accessed on 8 June 2020).

131 Mayne “What is low copy number (LCN) DNA typing?” *Hearsay*, <https://www.hearsay.org.au/what-is-low-copy-number-lcn-dna-typing/> (accessed on 25 September 2020).

132 Mayne “What is low copy number (LCN) DNA typing?” *Hearsay*, <https://www.hearsay.org.au/what-is-low-copy-number-lcn-dna-typing/> (accessed on 25 September 2020). See also Gill 2000:21.

2. The transfer of DNA to target surface may be due to the secondary transfer which can be identified only by thorough background and case investigation.
3. In case of first offenders, their profiles may not be available with the investigators for comparison and matching with the touch DNA profile.
4. Probable suspects should be available to match the touch DNA profile with their DNA profiles, so that the culprit can be linked to the crime scene, murder weapon and the victim.
5. Financial constraints associated with touch DNA technology should also be considered.
6. If these limitations are kept in mind, touch DNA can help in solving many criminal cases.¹³³

According to the Caddy Report, the following caveats also need to be borne in mind, namely:

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.¹³⁴

Budowle *et al.*¹³⁵ suggest that the following limitations of the LCN method should be disclosed by the prosecution. In the absence of such disclosure, these points raised by Budowle *et al.* could be pertinent to the construction of cross-examination questions which can impact on the weight/admissibility¹³⁶ of the evidence:

1. LCN typing is not a reproducible technique.
2. LCN results cannot be used to exclude an individual.
3. A concentrated sample may perform better in an analysis than replicates that use allele redundancy for interpretation.
4. The number and type of controls used should be defined and related confidence be provided quantitatively or qualitatively.
5. There are stochastic effects and the potential of contamination which impact LCN typing. The interpretation guidelines are not well-established, but those that exist are better suited for single-source samples. Mixture interpretation has not been validated.
6. Contamination or allele drop-in can come from several sources.

133 Sowmya 2016:25997.

134 Caddy *et al.* 2008:par. 7.4.

135 Budowle *et al.* 2009:214-215.

136 See above 4.1.

7. Due to the enhanced sensitivity, secondary transfer cannot be ruled out as a possible explanation for LCN typing results.
8. STR kits, some reagents, and other consumables may not have been subjected to sufficiently stringent quality control conditions to detect contamination from extraneous DNA similar to the rigor required for LCN typing
9. Statistical interpretations, and supporting data for probabilities, need to be better defined and developed to convey the uncertainty associated with LCN typing.
10. Because the analysis yields results from very minute samples, the tissue source of the DNA cannot currently be inferred.¹³⁷

The following recommendations to improve LCN DNA evidence, proposed by Word, could guide factfinders and legal practitioners in dealing with LCN evidence:¹³⁸

1. Conduct comprehensive validation studies of all techniques used in the laboratory, with particular focus on sensitivity, mixture and non-probative-sample studies, to develop stochastic thresholds and interpretation policies that accurately reflect the data obtained and the limitations of the test system.
2. Develop Standard Operating Procedures (SOP) very closely aligned with the procedures used in the validation studies.
3. Report what can be defended scientifically using report wording and statistical calculations that accurately reflect the data obtained without bias.
4. Make SOP, validation studies and electronic data (where printed profiles are inadequate for profile quality assessment) available in discovery.
5. Provide ample training to analysts regarding validation studies, procedures and policies, and interpretation of DNA profiles with limitations prior to beginning DNA casework.
6. Use caution to not “overinterpret” the data by recognizing that some samples may have insufficient data to definitively include or exclude an individual as a possible contributor, resulting in an “inconclusive” statement.

With regard to the question as to whether determining low template DNA profiles is a worthwhile exercise, Butler postulates that one needs to consider that success rates are often low; that the LCN process demands that

137 Budowle *et al.* 2009:214-215.

138 Word “What is LCN? – Definitions and challenges”, <https://worldwide.promega.com/resources/profiles-in-dna/2010/what-is-lcn-definitions-and-challenges/> (accessed on 24 May 2020).

contamination should be avoided or at least limited by the provision of sterile laboratories; that the complexity of the interpretation requires significant experience on the part of the analysts, and that the reliability of the significance of the match and the probative value of the findings may be affected by the fact that the time when the sample may have been deposited, cannot be determined.¹³⁹ However, despite these highly problematic aspects regarding attempts to establish LCN profiles, as summarised by Butler, the exposition in the article above, provides ample evidence that, in the absence of more accessible DNA material, and provided that the necessary precautions with regard to the interpretation of LCN evidence are taken, LCN profiles can be of immense value in combatting crime.

Lawyers and courts confronted with low level DNA samples should seriously consider the following observation by Stephen Breyer:

In this age of science, science should expect to find a warm welcome, perhaps a permanent home, in our courtrooms. The legal disputes before us increasingly involve the principles and tools of science. Proper resolution of those disputes matters not just to the litigants, but also to the general public – those who live in our technologically complex society and whom the law must serve. *Our decisions should reflect a proper scientific and technical understanding so that the law can respond to the needs of the public* (emphasis added).¹⁴⁰

In the final analysis, South African criminal courts and legal practitioners should pay heed to the observations outlined above, when the evidentiary value of LCN DNA arises for adjudication.

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