TRAFFIC

REPORT

# RhODIS® (RHINO DNA INDEX SYSTEM)

## Collaborative Action Planning Workshop Proceedings

FEBRUARY 2017

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TRAFFIC REPORT



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Front cover photograph and credit: White rhinoceros *Ceratotherium simum* adult and calf Southern Africa and East Africa. © Martin Harvey / WWF

**Recovering samples from rhinoceros horn for DNA extraction and analysis (©Veterinary Genetics Laboratory)**

# RhODIS®(RHINO DNA INDEX SYSTEM)

## Collaborative Action Planning Workshop Proceedings

*Ross McEwing, Nick Ahlers*



**Black rhinoceros** *Diceros bicornis* **adult and young in East Africa**

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the wildlife trade monitoring network

WWF

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YUNIBESITHI YA PRETORIA

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FROM THE AMERICAN PEOPLE

IUCN

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the wildlife forensics network

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# ABBREVIATIONS AND ACRONYMS

ARP ..... African Rhinoceros Programme

CITES.....Convention on International Trade in Endangered Species of Wild Fauna and Flora

ENFSI ..... European Network of Forensic Science Institutes

GEF.....Global Environment Facility

ISFG.....International Society of Forensic Genetics

IUCN..... International Union for the Conservation of Nature

KPN.....Kruger National Park

MOU ..... Memorandum of Understanding

NGO..... Non Governmental Organization

SWFS..... Society for Wildlife Forensic Sciences

UNDP .....United Nations Development Programme

UNODC..... United Nations Office on Drugs and Crime

USAID .....United States Agency for International Development

VGL ..... Veterinary Genetics Laboratory

WILDLIFE-TRAPS.....Wildlife Trafficking Response Assessment and Priority Setting Project

## LIST OF PARTNERS

### Organizers

**TRAFFIC** - the wildlife trade monitoring network, is the leading non-governmental organization working globally on trade in wild animals and plants in the context of both biodiversity conservation and sustainable development. TRAFFIC is a strategic alliance of IUCN and WWF.

**University of Pretoria** - The Onderstepoort Veterinary Genetics Laboratory at the Faculty of Veterinary Science, Onderstepoort is a service-driven and self-funding unit and is run on current and general business principles. It fulfils various roles including an academic support role for postgraduate students interested in applied veterinary genetics, research of genetic diseases in various animals and development of tests and protocols that can be applied in the service division.

**TRACE** - Wildlife Forensics Network is a not for profit organization based in the United Kingdom but working globally to promote the use of forensic science in wildlife crime prosecutions and wildlife trade monitoring through the delivery of training, capacity building and forensic test development projects.

### Donors

USAID - The United States Agency for International Development (USAID) is responsible for the majority of overseas development assistance from the United States government, including funding for international conservation. USAID works to end extreme poverty and promote resilient, democratic societies while advancing security and prosperity for America and the world.

USAID support for the workshop came through the Wildlife Trafficking, Response, Assessment and Priority Setting (Wildlife-TRAPS) Project. Implemented by TRAFFIC and IUCN, Wildlife-TRAPS combats wildlife trafficking through analyses and actions designed to secure a transformation in the level of co-operation among the international community of stakeholders impacted by illegal wildlife trade between Africa and Asia.

WWF - African Rhinoceros Programme (ARP) coordinates WWF's investment in rhinoceros conservation in Africa. It works closely with TRAFFIC, rhinoceros expert groups and WWF's various regional programmes and country offices. The ARP provides technical and leadership support to ensure overall coordination of rhinoceros-related projects.

## ACKNOWLEDGEMENTS

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# EXECUTIVE SUMMARY

The Wildlife Trafficking Response Assessment, and Priority Setting (Wildlife-TRAPS) project, supported by USAID and implemented by TRAFFIC and IUCN, organised in conjunction with the WWF African Rhinoceros Programme, University of Pretoria and TRACE Wildlife Forensics Network, a Collaborative Action Planning (CAP) workshop in Pretoria, South Africa between the 18th and 24th June 2016.

The workshop took place at the Berg en Dal Rest Camp in the Kruger National Park and at the Faculty of Veterinary Science of the University of Pretoria at Onderstepoort, Pretoria. It provided an opportunity for international scientists to interact and to experience the field conditions related to rhinoceros crime in South Africa, including the impact of poaching, crime scene sampling strategy and applied DNA testing methodologies. Delegates were introduced to the RhODIS® programme and how this could potentially be transferred and utilised on an international level. Each country presented their respective role and expertise in wildlife DNA forensic crime investigative techniques and shared their experiences. From an international perspective, consideration was given to the use of standardised forensic techniques for rhinoceros DNA testing. This aspect centred on the perceived need to internationalise the RhODIS® programme and to extend cooperative forensic DNA investigation of rhinoceros crime beyond South Africa.

Consensus was reached as to the role DNA forensics plays in the fight against rhinoceros related crimes and the requirement to better validate the RhODIS® system. Modalities to achieve this revolved around enhancing greater collaboration and cooperation between forensic laboratories and DNA experts from around the world. Law enforcement officials stressed the need to promote networking in regards to current challenges associated with rhinoceros wildlife crime highlighting areas for improvement in future case management.

After the five days of workshop discussions the following recommendations were proposed:

1. The analysis from DNA profiling of rhinoceros horn needs to be better utilised as a law enforcement tool. Currently, the results from analysing seized rhinoceros horn samples are not being disseminated effectively to inform trade data analysis or law enforcement investigations outside of South Africa. This highlights a lack of engagement, or developed network, to properly disseminate this information in the appropriate format for enforcement agencies or trade monitoring agencies to actively utilise such data. Efforts should be made to ensure that RhODIS® data is actively disseminated, where and when appropriate, to relevant monitoring and enforcement organisations and reports made documenting trade patterns to the CITES Secretariat.
2. An internationally standardised DNA species identification test for all rhinoceros species needs to be developed. The initial stage of identifying whether a suspected rhinoceros horn is actually from a rhinoceros, and if so, determining the rhinoceros species, so that the appropriate DNA profiling panel can be used to ascertain if the profile matches a carcass from the database. A mitochondrial cytochrome b gene test is the most useful and efforts should be made to develop a standardised system.
3. The RhODIS DNA system for Black (*Diceros bicornis*) and White (*Ceratotherium simum*) rhinoceros needs further validation. To ensure compliance with the legal systems in countries other than South Africa, additional validation requirements need to be addressed. Efforts should be made to rapidly publish the required validation data so the RhODIS® system can be utilised as widely as possible.
4. As a pilot study under a four year GEF-UNDP rhinoceros programme (2012 – 2016), an Environmental Forensic Section at the South Africa Police was established to develop police capacity for wildlife DNA forensic testing. A decision as to whether this section will be maintained, and to what extent it will manage the DNA testing, logistics and dissemination of rhinoceros DNA testing, is key to ongoing international testing and dissemination.

5. A DNA profiling system for the Greater One Horned rhinoceros (*Rhinoceros unicornis*), also known as the Indian rhinoceros, needs to be developed. As poaching and illegal trade is common in this species, and with a differing sentencing response in relation to animals poached within and outside national parks, the development of a DNA based individual identification technique could assist with enforcement operation in range countries of this species.
6. A mechanism to expedite the international transfer of samples from CITES-listed species for enforcement testing purposes needs to be developed. The inherent complexity of moving samples internationally for enforcement testing purposes presents challenges when DNA testing needs to be carried out rapidly. Efforts should be made to work with CITES to develop mechanisms where certain sample types can be fast tracked through the CITES process for the purposes of enhancing an illegal wildlife trade investigation.
7. Other forensic techniques to aid enforcement of the illegal rhinoceros horn trade require evaluation. Although DNA can be a powerful technique in wildlife crime investigation the technique can also be narrow in scope. Additional forensic techniques that can provide more information in relation to the perpetrators of crime should be investigated to add additional tools for enforcement officers to address illegal rhinoceros horn trade.

# INTRODUCTION

## Meeting Objective

The aim of the workshop was to promote enhanced collaboration and future cooperation between laboratories that provide forensic DNA testing of rhinoceros and support wildlife crime investigation in their respective countries and regions. Additionally, one of the initial category winners of the USAID 'Wildlife Crime Technology Challenge', the Veterinary Genetics Laboratory (VGL) at the University of Pretoria was seeking mechanisms to internationalise the RhODIS® DNA marker system for individual profiling of rhinoceros.

## Meeting Participants

To enable as fulsome and inclusive discussions as possible, delegates with specific enforcement or DNA technical expertise in this area were invited from rhinoceros range countries and non-range countries identified as key transit or consumer countries for rhinoceros horn. Delegates attended from South Africa, Kenya, Botswana, Namibia, India, Malaysia, Thailand, Indonesia, Viet Nam, Hong Kong, Korea, United Kingdom, Netherlands, Czech Republic and Australia, with additional representatives from TRACE, TRAFFIC, UNODC, USAID, the US Fish and Wildlife Service, and the World Bank. A full list of the participants is shown in Annex 1.

**Figure 1: Meeting participant group photograph at Kruger National Park (©Veterinary Genetics Laboratory)**

## Meeting Agenda

The meeting was divided into three sessions over five days (Annex II):

1. Presentation session from relevant experts in the field.
2. DNA Sampling and Data Management Session.
3. Workshop session to identify international issues relating to wildlife DNA forensics.

## Session 1. Presentations

The presentation session was split into two elements, the first being technical capacity presentations from rhinoceros range countries and non-range countries highlighting their current wildlife forensic DNA testing capabilities, and recent examples of rhinoceros-related casework where relevant (Annex III); the second session provided enforcement and forensic experts the opportunity to present on their work in this field.

Wildlife DNA Forensics laboratory capacity presentations were given by Kenya Wildlife Service, Police Forensic Laboratory (Botswana), Ministry of Environment and Tourism (Namibia), Wildlife

Institute of India, Eijkman Institute (Indonesia), Department of Wildlife and National Parks (Malaysia), Department of National Parks (Thailand), Hong Kong University / WWF (Hong Kong), National Institute of Biological Resources (Korea), Institute of Ecology & Biological Research (Vietnam), Charles University (Czech Republic), Australian Centre for Wildlife Genomics, and the Netherlands Forensic Institute.

Enforcement and rhinoceros DNA specific presentations were given by:

1. Nick Ahlers Project Leader of the Wildlife TRAPS Project, TRAFFIC. Background and overview of international wildlife trade.
2. Professor Adrian Linacre, Flinders University, Australia. Background and overview of wildlife DNA testing for forensic purposes and current trends.
3. Dr Cindy Harper, Director, Veterinary Genetics Laboratory, South Africa. RhODIS® and eRhODIS™.
4. Ms Frances Craigie, Chief Director Enforcement, Dept. Environmental Affairs. CITES issues and rhinoceros horn.
5. Dr Lucy Webster, National Wildlife DNA Forensic Laboratory, SASA, UK The use of rhinoceros DNA profiling in Europe to prevent zoo and rhinoceros horn thefts.
6. Dr Greta Frankham, Australian Centre for Wildlife Genomics. The development of rapid rhinoceros DNA species ID and its application in Vietnam.
7. Dr Ross McEwing, Director, TRACE Wildlife Forensics Network, UK. The challenges and benefits of international wildlife forensic cooperation.

## Session 2. Biological sampling and data management

Session 2 was divided into two components. The first component was a demonstration of the procedures and protocols developed by South African enforcement agencies for the collection of biological samples from stockpiled rhinoceros horn. The sampling was undertaken using the bespoke software Android App eRhODIS® developed by the Veterinary Genetics Laboratory which provides a basis for instruction, standard protocol for collection, and a platform for recording

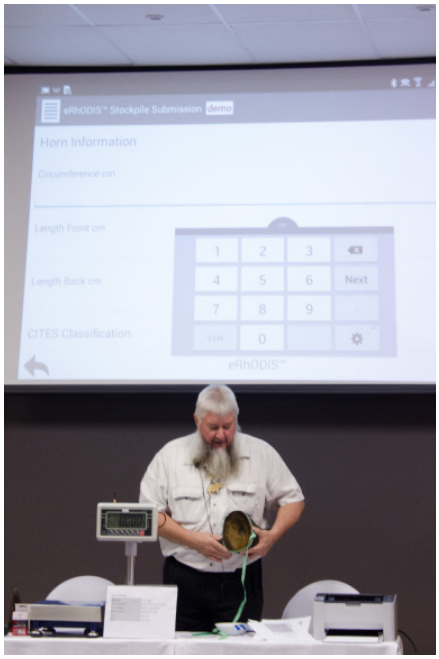


**Figure 2: Demonstration of rhinoceros stockpile procedures and the eRhODIS® App (©Simon Robertson)**

contemporaneously, metadata from a sample in a secure way to ensure the integrity of the biological samples as evidence, collected with a full chain of custody.
All international laboratory delegates were then provided with an opportunity, under direction, to take a sample from rhinoceros horn following the methodology developed by the Veterinary Genetics Laboratory; a practical element that could mimic sampling in their own country from seizures of suspected rhinoceros horn.

The second component for this session focused on collecting suitable biological samples from the field from a poached rhinoceros carcass. Given the frequency of rhinoceros poaching in Kruger National Park (an average of just under 3 animals per day) a suitable carcass (from a double rhinoceros poaching incident) was identified and the area was secured and forensically processed in advance to give all meeting delegates access and allow them to fully experience the challenges of the field. International laboratory delegates were again given the opportunity to carry out the sampling from the rhinoceros carcass and use the eRhODIS® Forensic App developed to be used in conjunction with DNA sampling kits to ensure the continuity of the evidence from a legal perspective. eRhODIS™ was developed as an adjunct for RhODIS® to aid in the collection of samples and information relevant to the RhODIS® project and is already in use in Namibia and Kenya.

**Figure 3: Poached rhinoceros briefing (©Simon Robertson)**

**Figure 4: One of the poached rhinos (©Simon Robertson)**

**Figure 5: Tissue sample from rhinoceros carcass (©Simon Robertson)**

**Figure 6: Ensuring chain of custody (©Simon Robertson)**

## Session 3. Developing international collaborations to improve enforcement of illegal rhinoceros horn trade through increased used of wildlife DNA forensic techniques

**Background to the RhODIS® system.**
RhODIS® is based on a set of DNA markers (microsatellites) originally developed for ecological research purposes[1] that, when combined, produce a DNA 'profile' from an individual. The uniqueness of that DNA profile, i.e. the likelihood that two individual DNA profiles are identical, is determined by the frequency of each variant (allele) of a DNA marker in a 'population' with the frequency of variants being established by DNA profiling a sufficient number of individuals from that 'population' which are expected to represent the population as a whole. The RhODIS® system is similar to other DNA profiling systems for individual identification used in a forensic context for other species[2].

RhODIS® can therefore be powerful at identifying 'linkages' between any two or more rhinoceros samples from an investigation, e.g. rhinoceros horn and an animal carcass; blood stained clothing and an animal carcass etc., by means of DNA profiling the biological samples and evaluating the likelihood that those biological samples are derived from the same individual.

The RhODIS® system incorporates a chain of custody procedure beginning at the scene of a crime, or evidence recovery point, and ending with a laboratory report identifying linkages between evidence items when appropriate.

Only by ensuring that a stringent chain of evidence exists, a robust set of DNA markers are utilised and a balanced interpretation of the data is reported, should DNA evidence be used with confidence in a legal framework.

The RhODIS® system has been used to generate evidence for prosecutions in South Africa that have led to convictions and custodial sentences. However, data on the number of cases where the system has been used is currently lacking; a not uncommon problem in the wildlife forensic field due to a disconnect between the prosecution department and laboratory service provider. This disconnect is often compounded when the service provider is not an agency of the government leading the investigation.

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1 C.A. Scott, Microsatellite variability in four contemporary rhinoceros species: implications for conservation, MSc Dissertation, Queen's University, Kingston, Ontario, Canada, 2008.
J. Cunningham, E.H. Harley, C. O'Ryan, Isolation and characterization of microsatellite loci in Black rhinoceros (*Diceros bicornis*) Electrophoresis, 20 (1999), pp. 1778–1780.
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L. Nielsen, D. Meehan-Meola, A. Kilbourn, A. Alcivar-Warren, Characterization of microsatellite loci in the Black rhinoceros (*Diceros bicornis*) and White rhinoceros (*Ceratotherium simum*): their use for cross-species amplification and differentiation between the two species Conserv. Gen., 9 (2008), pp. 239–242.
2 N. Dawnay, R. Ogden, R.S. Thorpe, L.C. Pope, D.A. Dawson, R. McEwing, A forensic STR profiling system for the Eurasian badger: a framework for developing profiling systems for wildlife species Forensic Sci. Int. Genet., 2 (2008), pp. 47–53.

# WORKSHOP ACTIVITIES

## Why internationalise the RhODIS® system?

The concept of internationalization of the RhODIS® system involves identifying other countries around the world that could utilise the markers, protocols and systems developed by the Veterinary Genetics Laboratory with the aim of standardising testing and better enforcing the illegal trade in rhinoceros horns.

Five possible drivers for internationalising the RhODIS® system were identified and discussed:

- Transit / consumer countries carrying out rhinoceros horn seizures provide additional evidence-based support for prosecutions back in South Africa.

In this scenario, countries would use the RhODIS® system to generate and provide DNA profile data from seized rhinoceros horns to South Africa, assuming this data could be legally accepted. As the seizure country would need to commit resources to enable this DNA profiling, such a scenario relies on an altruistic relationship which would in reality be difficult to implement and maintain. Additionally, it would seem unlikely that South Africa would try and implement extradition of those suspects involved in countries where seizures have taken place, particularly as enforcement actions should already be initiated in the country of seizure in relation to illegal trade offences.

- Transit / consumer countries implementing rhinoceros horn seizures to provide evidence to support prosecutions in their own country.

In this scenario, an additional evidence component would be produced that allowed a seized rhinoceros horn to be DNA profiled in another country, and where that DNA profile 'matched' a poaching carcass in a central database, this evidence would add weight to a prosecution in the country of seizure. Such a scenario would be effective in Black and White rhinoceros range countries where it would be analogous to the current South African system. However outside of Black and White range countries, such individual matching evidence is unnecessary and of little relevance to a prosecution where the prosecution aim is simply the identification of an illegally traded species.

- Agencies involved in monitoring and investigating transnational criminal networks.

In this scenario, DNA profiles from seized rhinoceros horns and data linking to poached individuals would be shared quickly with international organisations with a mandate to collect evidence of transnational illegal trade. Such data would then be used to profile criminal networks and initiate operations to disrupt the illegal trade. While there was general consensus that such a driver was potentially one of the most important uses of DNA profiling, it was acknowledged that, other than within the NGO community, such as TRAFFIC monitoring the illegal trade, there is no central coordination of law enforcement activities from a transnational perspective to incorporate this data at this time.

- Stockpile / seized rhinoceros horn management and auditing

In this scenario, DNA profiling is used by countries to monitor their seized rhinoceros horn or any stockpiles or other collections, e.g. museums / zoos, they hold to ensure those specimens are not laundered into the illegal trade. It was agreed that DNA registration systems for rhinoceros horn, similar to the system used in South Africa (Presentation 4) or Europe (Presentation 5) were useful but likely only where large collections of rhinoceros horn justify implementation.

- For the provision of data on trade routes.

In this scenario, DNA profiling from seized rhinoceros horns provides data, where it was able to be linked to a poached carcass, on the trade routes for illegal shipments of rhinoceros horn. Although such a system is currently encouraged through CITES, no data has been presented on trade routes from this process to date.

Therefore, as a tool for law enforcement, the internationalization of the current RhODIS® system would principally be to address national law enforcement within Black and White rhinoceros range countries outside South Africa, and as a tool for monitoring trade routes of illegal rhinoceros horn. Aspirationally, the system should be used to add information to enhance the profiling, and subsequent enforcement action, of individuals and criminal networks involved in the illegal rhinoceros horn trade, assuming central coordination of this activity could be initiated. A new DNA profiling system developed for the Greater One Horned rhinoceros, following the design of the RhODIS® system, would also be beneficial.

One important consideration is the cost / benefit of internationalizing the RhODIS® system, based on the above law enforcement drivers, against the current system where biological samples are sent to South Africa for DNA profiling. From 2009 until March 2014[4], only 10 rhinoceros horns were seized across eight non-range countries (Europe and USA); 61 rhinoceros horns across six transit/consumer countries in Asia (>50% in China, >26% Vietnam); and 77 rhinoceros horns seized in range countries (>68% from South Africa).

With such low numbers of individual seizures across countries, the argument for resourcing, developing capacity and maintaining the RhODIS® system in countries other than South Africa does not seem to be strong. Developing the RhODIS® system in China and Vietnam, both recognised transit and consumer countries for rhinoceros horn, could be justified. However, the lack of any identified central DNA testing laboratory in China (although DNA testing seems to be progressing in Hong Kong) and the limited capacity of the current Government mandated DNA testing laboratory in Vietnam limits implementation in these countries.

The development of a regional DNA testing facility[5] which could implement the RhODIS® system for neighbouring countries in a region lacking capacity, for example either Thailand or Malaysia acting as a testing hub in South-east Asia, could be an option. However, the same administrative burden with respect to transnational movement of samples currently exists whether samples are sent regionally or internationally to South Africa.

A simple solution to the drive for internationalization of the RhODIS® system could be the enhancement of the current DNA profiling in South Africa for rhinoceros, and its establishment as a global hub. Despite being the simplest and most cost-effective approach, it would require collaborative and coordinated engagement from South Africa, ensuring: i. that DNA testing was carried out to acceptable international standards; ii. that mechanisms for international sample transfer were simplified to expedite testing; and iii. that access to background DNA frequency data and carcass DNA profile data was accessible to, and therefore able to be interpreted by third country experts for legal presentation.

4 T. Milliken, Illegal trade in ivory and rhinoceros horn: an assessment report to improve law enforcement under the Wildlife TRAPS Project (2014) USAID and TRAFFIC.

5 R. Ogden, Forensic science, genetics and wildlife biology: getting the right mix for a wildlife DNA forensics lab. Forensic Sci. Med. Pathology, 6(3) (2010): 172-179.

## Requirements to internationalise the RhODIS® system and rhinoceros DNA forensics

The current RhODIS® system is specifically designed to DNA profile Black and White rhinoceros as it was established in South Africa, a range country for both species. However, at an international level, the illegal trade in rhinoceros horn also extends to Greater One-Horned rhinoceros and historically Javan (*Rhinoceros sondaicus*) and Sumatran (*Dicerorhinus sumatrensis*) rhinoceros which may still be traded illegally at a very low level. RhODIS® is able to distinguish Black from White rhinoceros based on fixed allelic differences but unable to distinguish other rhinoceros species. There was general agreement during the meeting that the development of a standardised DNA species identification test was the priority need from the international community where the requirement in any investigation is to determine whether a product is of rhinoceros origin, and to identify the species of rhinoceros to ascertain the likelihood of legality or otherwise. The consensus was that such a test should be a mitochondrial DNA Cytochrome b gene test, where the primers selected amplify a short fragment which could provide sufficient phylogenetic resolution while ensuring amplification from old material where the DNA would likely be fragmented. Work by the Australia Museums Laboratory (Presentation 6) highlighted their research in developing and testing new short fragment cytochrome b primer combinations specifically for rhinoceros species identification following DNA sequencing. It was agreed that, subject to some additional data requirements, these new primers should form the basis of a standardised species identification test for rhinoceros material.

The RhODIS® system has been used in legal cases in South Africa and therefore fulfils the legal requirements of that country. However, there were concerns raised at the workshop that the level of DNA marker validation was not sufficiently stringent for some countries to utilise this system as legal evidence (Presentation 4 & 5). It was agreed to develop a validation plan for RhODIS® that would fulfil the highest level of validation requirements so no country would have any concerns over the use of the system. This validation plan would address all recommendations developed by the Society for Wildlife Forensics (SWFS)[6], European Network of Forensic Science Institutes (ENFSI)[7] and the International Society of Forensic Genetics (ISFG)[8].

The issue of DNA quality was raised as a range of different extraction techniques for rhinoceros horn DNA were utilised by the international scientific delegates to the meeting. It was agreed that an example, but not obligatory, protocol should be developed to suggest the best procedure for extracting high yield, high quantity DNA from rhinoceros horn. Cost and availability of certain DNA extraction kits were the limiting factor in agreeing to develop a Standard Operating Protocol specifically for DNA extraction. Additional DNA extraction methodologies are currently being tested to evaluate their suitability with rhinoceros horn using chelex extractions (Presentation 6) and direct PCR (Presentation 2).

Assessing the quality of the DNA was deemed of high importance if DNA samples were to be sent to other laboratories around the world for DNA profiling. DNA profiling, unlike mtDNA gene sequencing, requires a higher yield of DNA to ensure successful and correct genotyping. Going to the effort of sending DNA samples without prior evaluation of the quality and quantity of DNA

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6 SWGWILD Standards and Guidelines (Version 2.0-Accepted by SWGWILD December 19, 2012).

7 Best Practice Manual for the Application of Molecular Methods for the Forensic Examination of Non-Human Biological Traces. ENFSI-BPM-APS-01 Version 01 (2015).

8 Linacre, A., Gusmão, L., Hecht, W., Hellmann, A.P., Mayr, W.R. ISFG: Recommendations regarding the use of non-human (animal) DNA in forensic genetic investigations. Forensic Sci. Int.: Genet. 2011;5:501–505.

being sent was considered pointless. A basic real-time PCR test was suggested as the simplest and most cost-effective DNA quality assessment method where DNA samples are amplified using a rhinoceros specific nuclear DNA target longer than the largest DNA profiling marker, and are run in parallel to an upper level concentration control sample and a lower level concentration control sample. Successful amplification of a DNA sample being tested, assessed by SYBR detection and subsequent melt curve product assessment as falling between those two controls, provides confidence that the DNA sample is of sufficient quality and quantity to undergo DNA profiling. A similar DNA quality assessment system developed for forest elephants was discussed earlier (Presentation 7).

The movement of biological samples between countries for the purposes of DNA testing was regarded as one of the main constraints to effective international collaborative approaches to DNA testing. There are two key issues that require addressing: the hazardous nature of biological samples; and the administrative burdens of CITES permitting.

Biological samples contain pathogens that can endanger human or animal health and therefore the international movements of biological material are restricted and closely controlled. For rhinoceros tissue or horn samples, such biological material would be classed as a 'Diagnostic Specimen' by the International Air Transporters Association (IATA) and require specific packaging and labelling. Individual countries have their own legislation and permitting procedures for allowing the importation of animal products. Purified, and treated, DNA extracts are likely to be easier to transport internationally, and more likely to be granted an import permit, if even required. DNA is likely to have been extracted from seized suspected rhino horn as part of the identification process, therefore assuming that the DNA extract meets the quality control criteria suggested above, it would be more appropriate to prioritise the transfer of DNA samples over rhinoceros horn samples, particularly when issues of lost rhinoceros horn samples during transportation had already been raised (Presentation 7). For South Africa, a certified master veterinary import permit must accompany all rhinoceros biological samples being sent in addition to a health certificate completed by the veterinary authority of the sending country.

CITES permits are required for all biological material, with the exception of faecal / urine samples, from all five extant rhinoceros species in respect to international transfer. Therefore, for the transfer of a single rhinoceros horn / DNA sample, the following applications and permits may be required: draft CITES import permit, CITES export permit, CITES import permit — assuming the sample is transferred directly between only two countries. The differences between the efficiency of different CITES Management and Scientific Authorities around the world is significant, and the time period to complete a permitting process for a sample transfer between two countries can be considerable (e.g. months) and therefore impede any active enforcement opportunities based on the results of DNA testing.

To alleviate the administrative and permitting burden on the scientific research community, CITES has implemented a 'Scientific Institution Status' where specific sample types of CITES-listed species from specific locations can be moved internationally without CITES import and export permits where both parties are registered with CITES, therefore expediting scientific research. However, the current CITES text covering this exemption is overly prescriptive of the specimens that can be transferred[9]. While some countries, e.g. United Kingdom, after lobbying (Presentation 7), have taken a more pragmatic view of this text[10], most countries still adhere strictly to the text as

9 Resolution Conf. 11.15 (Rev. CoP12).
10 General Guidance Note for The Use of Labels for Non-Commercial Loans by Scientific Institutions, Organisations or Individuals. GN16 April (2008) United Kingdom Animal and Plant Health Agency (APHA).

written and therefore this system is not particularly suitable for the transfer of enforcement related samples.

There has been an increasing reliance on forensic techniques to support enforcement and monitoring of illegal wildlife trade, but for the information produced by those tests to be relevant, it needs to be produced in a timely manner as dictated by the national laws in the country of seizure (or source country). The current systems that allow for the transfer of biological samples from CITES-listed species for forensic testing are often not effective for these time sensitive requirements.

Such sample transfers need to be rapid, auditable and reportable to CITES and perhaps be based on the transfer of samples between CITES registered 'Forensic Testing Laboratories' in a similar way to the Scientific Institution Status for academic researchers.

The requirement for rapid dissemination of results from testing was highlighted as an issue as there seemed to be a delay in getting access to rhinoceros horn samples to test, additionally there was an additional delay, or lack of clear protocol, in reporting the results of testing back to the country providing the samples.

This was highlighted (Presentation 7) in a case where Malaysia had provided 13 seized rhinoceros horn samples for DNA profiling to South Africa yet, despite the results matching White rhinoceros poached in South Africa, this information had not been reported back to the relevant Malaysian Government Authorities, despite the data being shared amongst African enforcement and NGO organisations. This lack of reporting is a key issue and shows a disconnect between the expectations of countries providing samples for testing, and resourcing that provision, and the current DNA testing laboratory in South Africa, that if not addressed, is likely to result in a lack of commitment or motivation to send future samples for testing.

There was a discussion around the reporting format of DNA profiling with some countries in a position to accept forensic reports generated by other countries, but most countries not able to accept the report as formal evidence in a prosecution. A case was highlighted back in 2011 (Presentation 7) where the DNA profiling of two White rhinoceros samples was undertaken in South Africa at the VGL laboratory, however the analysis of the results and evaluation of the subsequent 'match' was undertaken in the prosecuting country with a successful outcome. The need to explore the possibilities and options for international acceptance of results and data produced in other countries is key to the internationalization of RhODIS® as a law enforcement tool for prosecution.

Central to the acceptance of reports as evidence in a prosecution from an international perspective was the general agreement that all laboratories undertaking forensic DNA testing should be operating and be compliant with, but preferably accredited by, a Quality Assurance programme, for example ISO17025. Three of the wildlife forensic laboratories present at the meeting were accredited as ISO17025-compliant, and it was recommended that all laboratories need to be working toward these standards to ensure their laboratory and work are managed in a professional and quality assured way.

Although the majority of discussions focused around Black and White rhinoceros, there was a general consensus that a DNA profiling system is required for Greater One-Horned rhinoceros and that the development of such a system should harness the positive processes and tools developed by the RhODIS® system. It should also take into account the discussions from this workshop identifying some of the processes that should be addressed to make any DNA profiling system for Greater One-Horned rhinoceros a more comprehensive and internationally accepted test, an important consideration given the multiple range countries for this species.

# RECOMMENDATIONS AND ESSENTIAL NEXT STEPS

All of the delegates attending the workshop agreed that the illegal trade, historical or present, in rhinoceros horn was a significant threat to the long-term survival of all five rhinoceros species. Supporting enforcement action through wildlife forensic testing was recognised as a useful contribution to tackling the illegal trade but the following recommendations were identified as necessary actions to ensure best use of wildlife forensic techniques.

1. The analysis from DNA profiling of rhinoceros horn needs to be better utilised as a law enforcement tool. Currently, the results from analysing seized rhinoceros horn samples are not being disseminated effectively to inform trade data analysis or law enforcement investigations outside of South Africa. This highlights a lack of engagement, or developed network, to properly disseminate this information in the appropriate format for enforcement agencies or trade monitoring agencies to actively utilise such data. Efforts should be made to ensure that RhODIS® data is actively disseminated, where and when appropriate, to relevant monitoring and enforcement organisations and reports made documenting trade patterns to the CITES Secretariat.
2. An internationally standardised DNA species identification test for all rhinoceros species needs to be developed. The initial stage of identifying whether a suspected rhinoceros horn is actually from a rhinoceros, and if so, determining the rhinoceros species, so that the appropriate DNA profiling panel can be used to ascertain if the profile matches a carcass from the database. A mitochondrial cytochrome b gene test is the most useful and efforts should be made to develop a standardised system.
3. The RhODIS DNA system for Black and White rhinoceros needs further validation. To ensure compliance with the legal systems in countries other than South Africa, additional validation requirements need to be addressed. Efforts should be made to rapidly publish the required validation data so the RhODIS® system can be utilised as widely as possible.
4. As a pilot study under a four year GEF-UNDP rhinoceros programme (2012 – 2016), an Environmental Forensic Section at the South Africa Police was established to develop police capacity for wildlife DNA forensic testing. A decision as to whether this section will be maintained, and to what extent it will manage the DNA testing, logistics and dissemination of rhinoceros DNA testing, is key to ongoing international testing and dissemination.
5. A DNA profiling system for the Greater One Horned rhinoceros, also known as the Indian rhinoceros, needs to be developed. As poaching and illegal trade is common in this species, and with a differing sentencing response in relation to animals poached within and outside national parks, the development of a DNA based individual identification technique could assist with enforcement operation in range countries of this species.
6. A mechanism to expedite the international transfer of samples from CITES-listed species for enforcement testing purposes needs to be developed. The inherent complexity of moving samples internationally for enforcement testing purposes presents challenges when DNA testing needs to be carried out rapidly. Efforts should be made to work with CITES to develop mechanisms where certain sample types can be fast tracked through the CITES process for the purposes of enhancing an illegal wildlife trade investigation.
7. Other forensic techniques to aid enforcement of the illegal rhinoceros horn trade require evaluation. Although DNA can be a powerful technique in wildlife crime investigation the technique can also be narrow in scope. Additional forensic techniques that can provide more information in relation to the perpetrators of crime should be investigated to add additional tools for enforcement officers to address illegal rhinoceros horn trade.

## Additional Comments

Three additional issues were raised at the meeting to further help law enforcement efforts to combat illegal trade in rhinoceros horn.

1. The development of a photographic guide to rhinoceros horn to aid with identification from very small fragments of horn. Such small fragments have been used to produce full DNA profiles by VGL but there was difficulty in separating rhinoceros horn fragments from other, possibly inhibiting, organic matter.
2. While DNA isolated from rhinoceros horn is a powerful technique, additional forensic techniques such as human fingerprint recovery and human touch DNA should not be ignored as they may also provide valuable information on persons involved in the illegal trade.
3. The commercial production of synthetic rhinoceros horn was raised as a concern as there remains uncertainty as to the production methods, or how such products could be separated from real rhinoceros horn, and therefore whether these products could cause law enforcement identification challenges.

# ANNEXES

## Annex 1: Workshop Programme

| Time | Activity |
|---|---|
| Throughout Day | Arrival of delegates |
| Before 10:00 | Arrival of remaining delegates |
| **Day 1** | |
| 10:00 – 15:00 | Travel to Kruger National Park (KNP) by Road and arrival at Berg en Dal Camp |
| 15:00 – 16:00 | Break |
| 16:00 – 16:30 | Welcome and Introductions – Prof Alan Guthrie |
| 16:30 – 20:30 | Game Drive followed by Welcome Braai |
| **Day 2** | |
| 07:30 – 08:30 | Breakfast |
| 08:30 – 08:45 | Welcome and Overview of Workshop Agenda - Dr Joseph Okori |
| 08:45 – 09:05 | Background and overview of International Wildlife Trade - Nick Ahlers- TRAFFIC |
| 09:05 – 09:45 | Background and overview of wildlife DNA testing for forensic purposes and current trends - Prof Adrian Linacre |
| 09:45 – 10:00 | Delegate Laboratory Presentations |
| 10:00 – 10:15 | Coffee Break |
| 10:15 – 12:30 | Delegate Laboratory Presentations Continued |
| 12:30 – 13:30 | Lunch |
| 13:30 – 14:00 | Delegate Laboratory Presentations Continued |
| 14:00 – 14:40 | RhODIS®and eRhODIS™ - Cindy Harper |
| 14:40 – 15:00 | CITES issues and rhinoceros horn - Frances Craigie |
| 15:00 – 15:25 | The use of rhinoceros DNA profiling in Europe to prevent zoo and rhinoceros horn thefts. - Dr Lucy Webster |
| 15:25 – 15:40 | Tea Break |
| 15:40 – 16:00 | Presentation on the development of rapid rhinoceros DNA species ID and its application in Vietnam. - Dr Greta Frankham |
| 16:00 – 16:20 | The challenges and benefits of international wildlife forensic cooperation. - Dr Ross McEwing |
| 16:20 – 16:35 | Break |
| 16:35 – 18:00 | Possible Game Drive |
| 19:00 | Dinner |
| **Day 3** | |
| 06:30 – 08:30 | Travel to Skukuza Camp, KNP<br>08:30 - 09:30<br>Short background to rhinoceros poaching problem, efforts and strategy in KNP and KwaZulu-Natal (KZN) and Asia (India and Nepal Representatives |

| Time | Activity |
|---|---|
| 09:30 – 14:00 | Visit a rhinoceros carcass and field sampling for DNA, use of sampling kits |
| 14:00 – 15:00 | Break/ Late Lunch at Skukuza Camp |
| 15:00 – 17:00 | Travel back to Berg en Dal Camp |
| 19:00 | Dinner |
| **Day 4** | |
| 07:30 – 08:30 | Breakfast |
| 08:30- 16:00 | Travel back to Pretoria with stop at Pilgrims Rest (TBD) |
| 16:00 – 17:00 | Settle into accommodation at the Visiting Scientists Apartments, University of Pretoria |
| 17:00 – 17:30 | Introduce workshop items to be discussed on Thursday |
| 19:00 | Dinner |
| **Day 5** | |
| 07:30 – 09:00 | Breakfast |
| 09:00 – 09:15 | Overview of the day and technical working groups |
| 09:15 – 10:00 (includes plenary feedback session) | Activity 1: Establish requirements DNA support for domestic PROSECUTION – relating to rhinoceros samples<br>Breakout groups –<br>a. Rhinoceros range states<br>b. Non range states |
| 10:00 – 10:45 | Activity 2: Do we as wildlife DNA forensic scientists have sufficiently robust DNA assays to address PROSECUTION questions.<br>Breakout groups –<br>a. All DNA technical delegates |
| 10:00 – 10:45 | Activity 3: Do we as monitoring / intelligence gathering agencies have requirements to capture DNA data from rhinoceros material?<br>Breakout groups –<br>a. All non DNA technical delegates |
| 10:45 – 11:15 | Plenary feedback from Activity 2 & 3 |
| 11:15 – 11:45 | Coffee Break |
| 11:45 – 12:30 | Plenary Activity 4. What are the barriers for countries in providing specific DNA data from seizures to monitoring / intelligence gathering agencies? |
| 12:30 – 13:30 | Lunch |
| 13:30 – 15:00 Plenary | Activity 5: What are the work packages to be developed resulting from the workshop? |
| 15:00 – 15:15 | Tea Break |
| 15:15 – 16:15 Plenary | Activity 6: Do these work packages align with existing donor funding / activities |
| 16:15 – 16:30 | Key Summaries from Group Discussions and Next Steps |
| 16:30 – 18:30 | Tour of RhODIS Lab- suggestion Southeast Asia delegates go first |
| 19:00 | Dinner |

| Time | Activity |
|---|---|
| **Day 6** | |
| 07:30 – 09:00 | Breakfast |
| 09:00 – 12:00 | Remaining delegates tour RhODIS lab<br>Southeast Asia Delegates take tour of Professor Antoinette's lab at Pretoria Zoo for discussion on MOU for sharing DNA sequence data |
| 12:00 – 13:00 | Lunch |
| Afternoon/ Evening | Delegates depart |

# Annex 2: Participants' List

| Name | Country | Institution |
|---|---|---|
| Jorge Rios | USA | UNODC |
| Simon Robertson | USA/UK | WORK BANK GROUP |
| Ed Newcomer | Botswana | US Fish and Wildlife |
| Major Steve Roets | South Africa | South African Police Services |
| Col Johan Jooste | South Africa | South African Police Services |
| Lt Francois Vemaak | South Africa | South African Police Services |
| Ken Chan | Hong Kong | WWF Hong Kong |
| Vicki Sheng | Hong Kong | Hong Kong University |
| Jumin Jun | Korea | Wildlife Genetic Resources Centre |
| Kyle Ewart | Australia | Australian Centre for Wildlife Genomics |
| Dr Greta Frankham | Australia | Australian Centre for Wildlife Genomics |
| Dr Phuong Trang | Vietnam | IEBR CITES SA |
| Dr The Dang tat | Vietnam | IEBR CITES SA |
| Jessica Wiludjaja | Indonesia | Eijkman Institute |
| Dr Kanitia Ovouthan | Thailand | Department of National Parks, Thailand |
| Ms Kitichaya Penchart | Thailand | Department of National Parks, Thailand |
| Dr Jeffrine Rovie | Malaysia | Department of Wildlife and National Parks, Malaysia |
| Frankie Sitam | Malaysia | Department of Wildlife and National Parks, Malaysia |
| Dr Magora | Botswana | Botswana Police Service |
| Mr Waale | Botswana | |
| Lucy Webster | United Kingdom | Scottish Agriculture (SASA) |
| Moses Otiende | Kenya | Kenya Wildlife Services Forensic Laboratory |
| Linus Kariuki | Kenya | Kenya Wildlife Services |
| Prof George Owiti | Kenya | University of Nairobi-Faculty of Veterinary Medicine |
| Prof Adrian Linacre | Australia | Flinders University Australia |
| Dr Cindy Harper | South Africa | University of Pretoria |
| Prof. Alan Guthrie | South Africa | University of Pretoria |

| Name | Country | Institution |
|---|---|---|
| Dr Samrat Mondol | India | Wildlife Institute of India |
| Nick Ahlers | South Africa | TRAFFIC |
| Dr Ross McEwing | United Kingdom | TRACE Wildlife Forensics Network |
| Dr Richard Emslie | South Africa | IUCN Africa Rhinoceros Specialist Group |
| Rod Potter | South Africa | KZN |
| Dr Joseph Okori | South Africa | WWF African Rhinoceros Programme |
| Pavla Rihova | CZECH REPUBLIC | Czech Environmental Inspectorate |
| Dr Daniel Vanek | CZECH REPUBLIC | DIRECTOR FORENSIC DNA SERVICE |
| Dr Irene Krupper | Netherlands | Netherlands Forensics Institute |
| Frances Craigie | South Africa | Ministry of Environmental Affairs |
| Sonja De Klerk | South Africa | South African Police Services |
| Dr Carlos Lopes Pereira | Mozambique | Mozambique government |
| Chantel Louw | Namibia | Ministry of Environment and Tourism |
| Birgit Kotting | Namibia | Ministry of Environment and Tourism |
| Dr Rob Ogden | United Kingdom | Society for Wildlife Forensic Science |

## Annex 3: Current capacity of range and non-range states in relation to rhinoceros DNA testing

| Country | Range state | Species | Central Lab | Lab Type | Sp. ID | Geo. origin | Indiv. ID | Comments |
|---|---|---|---|---|---|---|---|---|
| South Africa | Y | W,B | Y | U & G | Y | Y | Y | RhODIS started here, eRhODIS developed to improve collection process; only Black and White rhinoceros at this stage |
| Kenya | Y | W,B | Y | G | Y | Has capacity | Has capacity | DNA evidence accepted in court; Adopted RhODIS, send samples to VGL; MOU between University of Pretoria and Kenya to protect data |
| Botswana | Y | W,B | N | G | Y | | | Lab in Gabarone has limited but developing capacity for wildlife crime – all samples sent to NZG in South Africa. Challenge of moving samples within Botswana due to foot-and-mouth disease in the NW where most of the crimes occur and lab in the NE (not in foot-and-mouth area); when samples are sent to SA, send staff to learn |
| Namibia | Y | W,B | N | | | | | Send all to SA; delays with permits (SA side); have a human lab, would like to expand to wildlife; also need to develop capacity within the police for wildlife crime |
| India | Y | I | Y | G | Y | Has capacity | Has capacity | Approved RhODIS system, funding for next 3 years this will add geographic origin and Indiv. ID testing that will be useful for legal cases |

| Country | Range state | Species | Central Lab | Lab Type | Sp. ID | Geo. origin | Indiv. ID | Comments |
|---|---|---|---|---|---|---|---|---|
| Indonesia | Y | J, S | Y | G | Y | Has capacity | Has capacity | Only had one international rhinoceros case so far and it was water buffalo<br>Need genetic database to support conservation strategies (STRs and mtDNA) |
| Malaysia | N | | Y | G | Y | Has capacity | Has capacity | Major transit country. Well developed wildlife forensic laboratory capacity to do STR, SNPs and DNA sequencing |
| Thailand | N | | Y | G | Y | Has capacity | Has capacity | Transit country. Has capacity to do STRs for geographic origin and individual ID |
| Hong Kong | N | | N | U | Y | | | Global epicentre for wildlife trade – gateway to Chinese market. Centralised lab facility needed. Currently results only used for intelligence gathering, not in court |
| Korea | N | | Y | G | Y | Has capacity | Has capacity | Involved in forensic cases, but no recent rhinoceros cases so far |
| Vietnam | N | | Y | G | Y | | | Want to develop capacity and knowledge for geographic origin/subpopulation testing |
| Czech Republic | N | | N | U | Y | Has capacity | Has capacity | Centre of illegal wildlife trade in Europe; Forensic DNA testing lab at University. Has sent rhinoceros samples to VGL – result used for intelligence gathering only – report not recognised in Czech court. |
| Australia | N | | | U | Y | Has capacity | Has capacity | ISO17025 certified lab for molecular diagnostic services; accredited tissue bank; lots of research and outreach |

| Country | Range state | Species | Central Lab | Lab Type | Sp. ID | Geo. origin | Indiv. ID | Comments |
|---|---|---|---|---|---|---|---|---|
| United Kingdom | N | | Y | G | Y | Has capacity | Y | Working with museum and zoo samples on Black, White and Greater One Horned rhinoceros using same markers as RhODIS |
| Netherlands | N | | Y | G | Y | Has capacity | Has capacity | Capacity development with Botswana; also do aging of rhinoceros horn |

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TRAFFIC, the wildlife trade monitoring network, is the leading non-governmental organization working globally on trade in wild animals and plants in the context of both biodiversity conservation and sustainable development.

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