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Defining end user requirements for a field-based molecular detection system for wildlife forensic investigations

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Abstract

The increasing use of non-laboratory-based DNA and protein detection methods promise to provide rapid investigative intelligence and support sample prioritisation. Primarily developed for human forensic or medical applications, current systems may also show utility in the field of wildlife forensic science. However, it is currently unknown whether the requirements of the wildlife forensic community can be met by current non-laboratory based tools. Given the diverse array of stakeholders and sample types commonly encountered, it is necessary to first identify the needs of the community and then try and map their needs to current instrumentation. By using a market research style questionnaire, this study identified key requirements for a non-

laboratory-based system following feedback from the wildlife forensic community. Data showed that there is strong support for field-based detection methods while highlighting concerns including contamination risks and reduced quality assurance associated with non-laboratory testing. Key species and applications were identified alongside hurdles to implementation and adoption. Broadly, the requirements align with many of the developmental drivers that have led to the rise of in-field portable detection instrumentation, specifically rapid detection within one hour, ease-of-use, and ≥95% accuracy. Several existing platforms exist that met some of the identified requirements but not all. With further collaboration between industry partners and the wildlife forensic community it is possible that new field-based systems can be developed and applied routinely.

Key words: *Field-based testing; molecular; wildlife; forensic; industry; development*

1. Introduction

The illegal wildlife trade (IWT) poses a huge threat to the survival of many species. The black-market trade in endangered species is estimated between US$5 billion and US$20 billion a year and disrupting the trade requires a multi-faceted approach [1, 2]. Challenges in understanding IWT include the covert and transnational nature of the trade [3], coupled with difficulties associated with discovering and then identifying illicit items by non-specialist regulatory officers [4]. This is typically achieved using traditional investigative approaches, such as intelligence-led international operations [5, 6], or through random searches of items at borders [7]. Confirming the species identity of seized items, or determining whether or not they contain derivatives of an endangered species, is then necessary to support a criminal prosecution [8]. However, given the heavily processed nature of many of the samples recovered, or the lack of species distinguishing characters between immature specimens of many species, diagnostic

identification needs to be performed. Currently this is conducted by specialist laboratories with expertise in morphological or molecular identification techniques [9-11], but the development and future implementation of field-based analytical equipment may allow on-site-analysis saving both time and investigative resources.

Portable rapid detection methods can detect either DNA or proteins unique to the sample of interest and be developed to match the end-user requirements depending on the field of research. The potential for application in forensic science has long been recognised by the human forensic community, where consultation with stakeholders has revealed a number of clearly defined end user requirements [12, 13]. These requirements have allowed industry groups to develop and commercialise several DNA and immunoassay approaches [e.g. 14-17]. Such advancements now allow analysis of forensically relevant samples by police officers and Crime Scene Investigators out of the laboratory. While a large proportion of this work has focussed on human forensic applications, there is evidence that similar approaches may be useful in the wildlife forensic arena [18-20]. However, the application of such portable approaches to wildlife forensics is likely to be complicated by the diverse array of sample types encountered in casework and the ability of any of the existing instrumentation to fulfil the requirements of the end user. Furthermore, the timeframe for development, validation and implementation of any approach in a wildlife forensic context is very difficult to predict given the diverse array of jurisdictions and the individual needs of specialist forensic groups. It is therefore possible that for the foreseeable future field-based approaches are restricted to presumptive test applications, complimenting subsequent confirmatory analysis at a laboratory; that said, it seems likely that wildlife forensic applications will reach the field at some point.

This study seeks to identify the key requirements of a field-based detection system as required by potential end users and wider stakeholder groups in the wildlife forensic and law enforcement arena. In doing so, the community's needs can either be mapped to identify a

compatible instrument or the need for more bespoke instrumentation and support from industry developers.

2. Methodology

An online questionnaire (supplemental material 1) was distributed using SurveyMonkey Inc (San Mateo, California, USA) to participants at the 2017 Society for Wildlife Forensic Science (SWFS) conference in Edinburgh and to postgraduate students studying at the Liverpool Centre for Advance Policing (LCAP). The survey was voluntary, anonymised and no personal information was collected. The research was granted ethical approval prior to being conducted (Approval Number 17/PBS/004).

In total, 100 individuals participated in the survey; 78 SWFS participants and 22 LCAP participants. Average completion rate of the questionnaire was 74%. Response data was exported to Excel and weighted averages applied to all rank questions. Preliminary analysis allowed the grouping of individuals into four broad categories based on their profession; **laboratory-based researcher** (n=27; consisting of university or government researchers), **laboratory-based practitioner** (n=25; consisting of scientists employed to provide analytical services, e.g. forensic caseworkers, food standards, conservation), **field-based practitioner** (n=35; consisting of customs/border control, field-based wildlife crime investigators, police/enforcement officers and postgraduate students in policing and criminal investigation) and **desk-based individuals** (n=13; consisting of charity/NGO/policy representatives and R&D project managers).

4. Results and Discussion

4.1. Stakeholder Awareness

The data shows a knowledge gap may exist between user groups regarding awareness of field-based DNA systems (Table 1A). The data shows that ~68% of field-based practitioners have 'some' or 'very little' knowledge of current field-based detection systems compared to ~50% of desk-based individuals who described themselves as being 'very familiar' or 'familiar'. A similar proportion was also seen in the lab-based practitioner group, ~48% of whom also identified as being 'very familiar' or 'familiar' while the most aware were the lab-based researchers, ~67% of whom were 'very familiar' or 'familiar' with current field based systems. One possible explanation for the lack of familiarity observed in the field-based practitioner group is that many of the field-based systems are only recently out of the R&D phase. As such, much of the information available has been disseminated through scientific publications with little targeted knowledge transfer to field-based end-users. Similar knowledge gaps have been reported between the enforcement and research communities with other technology [21, 22], and has been cited as a reason for the slow adoption of pioneering research by enforcement groups.

Table 1. *Ranking of the issues preventing the use of field-based instrumentation in wildlife forensic casework and participant's level of familiarity with current, field-based DNA instruments.*

Topic under evaluation and response options	Field-based Practitioner		Lab-based Practitioner		Desk-based Individual		Lab-based Researcher		All Participants	
A) Participants level of familiarity with field instrumentation	**Percent (%)**									
1) Very familiar with current, field-based DNA instruments	0.0		20.0		16.7		25.0		12.6	
2) Familiar with some platforms	14.7		28.0		33.3		41.7		26.3	
3) Some literature-based knowledge	35.3		40.0		25.0		8.3		30.5	
4) Very little known	32.4		8.0		20.8		16.7		21.1	
5) Not previously aware of field-based DNA instrumentation	17.6		4.0		4.2		8.3		9.5	
B) Issues preventing the use of field-based instrumentation	**Weighted average of the scores (rank)**									
1) Cost	**2.15**	**(1)**	**1.46**	**(1)**	**0.80**	**(1)**	**1.66**	**(1)**	**6.07**	**(1)**
2) Lack of funding for purchasing	**1.89**	**(2)**	1.26	(4)	**0.73**	**(2)**	**1.35**	**(2)**	**5.23**	**(3)**
3) Accuracy of the test and instrument	**1.80**	**(3)**	**1.44**	**(2)**	**0.68**	**(3)**	**1.35**	**(2)**	**5.28**	**(2)**
4) Sensitivity of the test and instrument	1.54	(6)	**1.46**	**(1)**	0.65	(4)	**1.24**	**(3)**	4.89	(4)
5) Lack of an instrument that suits my needs	1.77	(4)	**1.30**	**(3)**	0.41	(7)	1.20	(4)	4.68	(5)
6) Lack of an assay that I can use	1.28	(7)	1.18	(5)	0.44	(6)	1.12	(5)	4.02	(7)
7) Ease of use	1.59	(5)	1.12	(6)	0.51	(5)	0.89	(6)	4.11	(6)
8) The colour of the instrumentation	0.72	(8)	0.43	(7)	0.16	(8)	0.40	(7)	1.71	(8)

A - Results are the calculated percentage of participants (%) based on the number of responders. Number of responders to question was 34 for field-based practitioner, 25 for lab-based practitioner, 12 for desk-based individual, 24 for lab-based researcher.

B - Results were ranked using a weighted average of the scores (1-8) entered by participants giving more importance to the issues selected first. Number of responders to question was 30 for field-based practitioner, 22 for lab-based practitioner, 10 for desk-based individual, 21 for lab-based researcher.

4.2. Perceived issues regarding the adoption of field-based instrumentation

Participants from all groups selected 'cost' as the primary issue preventing the adoption of field-based instrumentation (Table 1B). Regarding the maximum per sample cost of analysis the data reveals that 2% of participants would consent to paying £100 per sample; 14% would pay £50 per sample; 37% would pay £20; 32% would pay £10; while 14% identify £1 as the maximum per sample analysis cost. Together the data suggests that a consumable cost of £20 per sample will satisfy 53% of users. With regards to maximum instrumentation cost, the data shows that none of the participants would pay £100,000 for a field-based detection system; 3% would pay £50,000; 16% would pay £10,000; 41% would pay £5,000; 26% would pay £1,000; while only 14% of are looking for instrumentation that costs £100. Together the data suggests that an instrumentation cost of £5,000 per unit will satisfy 60% of surveyed users. Results indicate that assay and instrument cost are key issues for commercial development groups to consider if they want to expand into the wildlife forensic marketplace. Data also shows that the funding needed to purchase such instrumentation would be secured from a variety of different sources; 42% from government grants; 27% from academic funding bodies; 15% from internal institutional based funding calls; and 15% from NGO or charity funding. The emphasis on central government financing suggests there may be a need for specific funding to facilitate the adoption of field-based instrumentation.

The second overall hurdle to implementing field-based testing as a strategy was the instrument and test 'accuracy' (Table 1B). Analysis shows that 67% of respondents would be satisfied with a test accuracy of 95%, while only 33% of participants require a test with 99-100% accuracy (Figure 1a). Test accuracy is a measure of the agreement between the 'information' obtained from the sample under evaluation and a controlled standard or voucher specimen. The type of 'information' provided will depend on the purpose of the test (see section 4.4 below), although diagnostic accuracy can be expressed in many ways, including '*Sensitivity'* and '*Specificity'*

[23, 24]. Under this definition, the number of True Positives, False Positives, True Negatives and False Negatives are recorded. These numbers are used to report on the test *Sensitivity* (the proportion of true positives that are correctly identified by the test) and *Specificity* (the proportion of true negatives that are correctly identified by the test) with very accurate tests show a high percentage scores for both. The number of false negatives recorded can vary as a function of the system's Limit of Detection (LOD) and reduce the overall measure of accuracy. The data in Figure 1b shows that 4% of participants suggest an LOD of ≤ 1000 cells; 16% suggest LOD of ≤ 500 cells; 43% suggest an LOD of 100 cells; while 37% suggest an LOD of <10 cells. Together, the data shows that 63% of respondents consider detection of ≤ 100 cells an acceptable LOD. This is largely in line with the limit of detection displayed by human forensic tests. One explanation for the different requirements is that each stakeholder group likely process different types of biological sample, ranging from DNA rich items such as tissue and blood to extremely low concertation samples such as powdered derivatives or trace material.

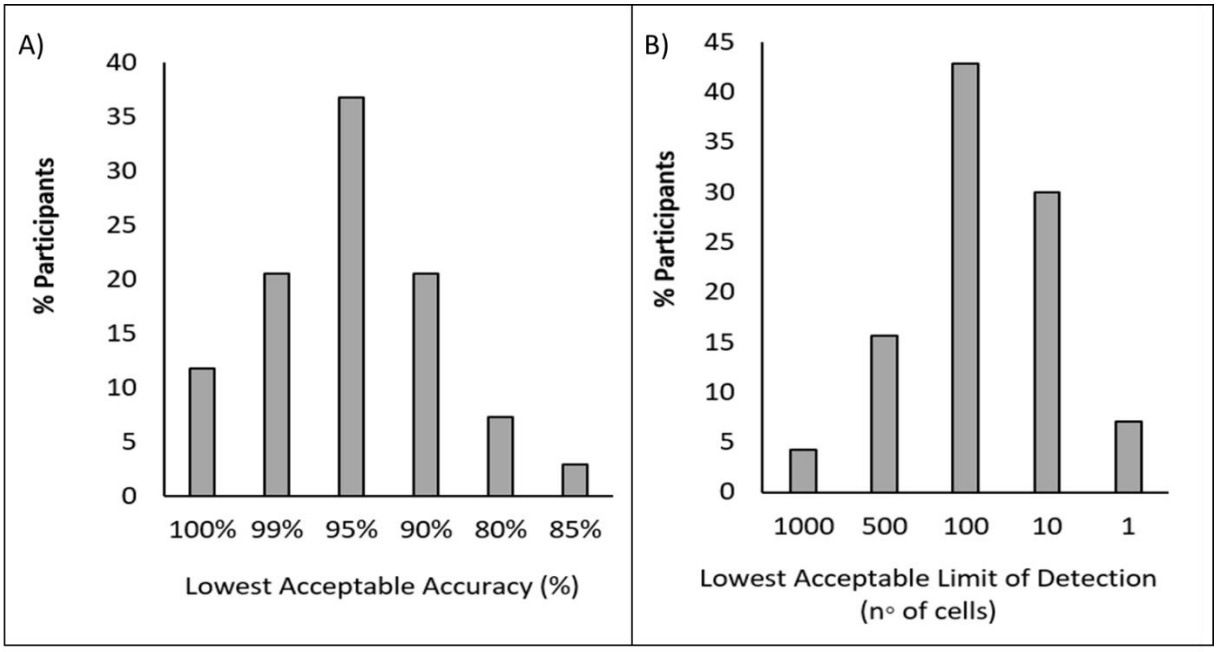

Figure 1. *Test Accuracy (a) was identified as a hurdle to implementing field-based systems together with the test Limit of Detection (b). Number of responders to question was 26 for field-based practitioner, 14 for lab-based practitioner, 12 for desk-based individual, and 18 for lab-based researcher.*

Portable rapid detection tests are typically described as either being '*presumptive*' or '*diagnostic*'. Presumptive tests will produce a higher number false positive and false negative results and are therefore less accurate than diagnostic tests used in the laboratory [12, 25]. There is no strict classification of what is required to classify a test as being either presumptive or diagnostic based on its accuracy although the data suggests that there is room for the development of presumptive tests with 95% accuracy at 100 cells input which may include affordable and easy-to-use immunoassay-based approaches [e.g. 18] as well as more sensitive and specific DNA based approaches [e.g. 19, 20].

Other highly ranked issues included increasing contamination events as PCR moves out of the laboratory and a reduction in QA/QC as processes become field-based (Figure 2). These represent serious concerns to the adoption of field-based testing even when using tests with a high reported accuracy as the QA/QC practices of a testing laboratory may differ markedly from the processes employed at a crime scene, in the field, or at boarders. However, it should be recognised that the necessity to adopt ISO17025 standards during sample collection is not unique to wildlife forensic investigations as both Crime Scene Investigators and Sexual Assault Referral Centre Staff handling human casework samples have only in the last few years begun adopting and defining sector specific standards [26]. As such, it is considered likely that the wildlife community follow suit and that training and knowledge transfer events be organised in preparation for the adoption of field-based testing supported by community working groups, government regulators and special interest groups. Such training will need to also look at the validation of the novel technology prior to use in forensic investigations. The validation process is universally recognised by laboratory analysists and validation guidelines and recommendations are available [26, 27]. However, with field-based technology the responsibility for validation will fall on the shoulders of enforcement teams who may have little experience in this area.

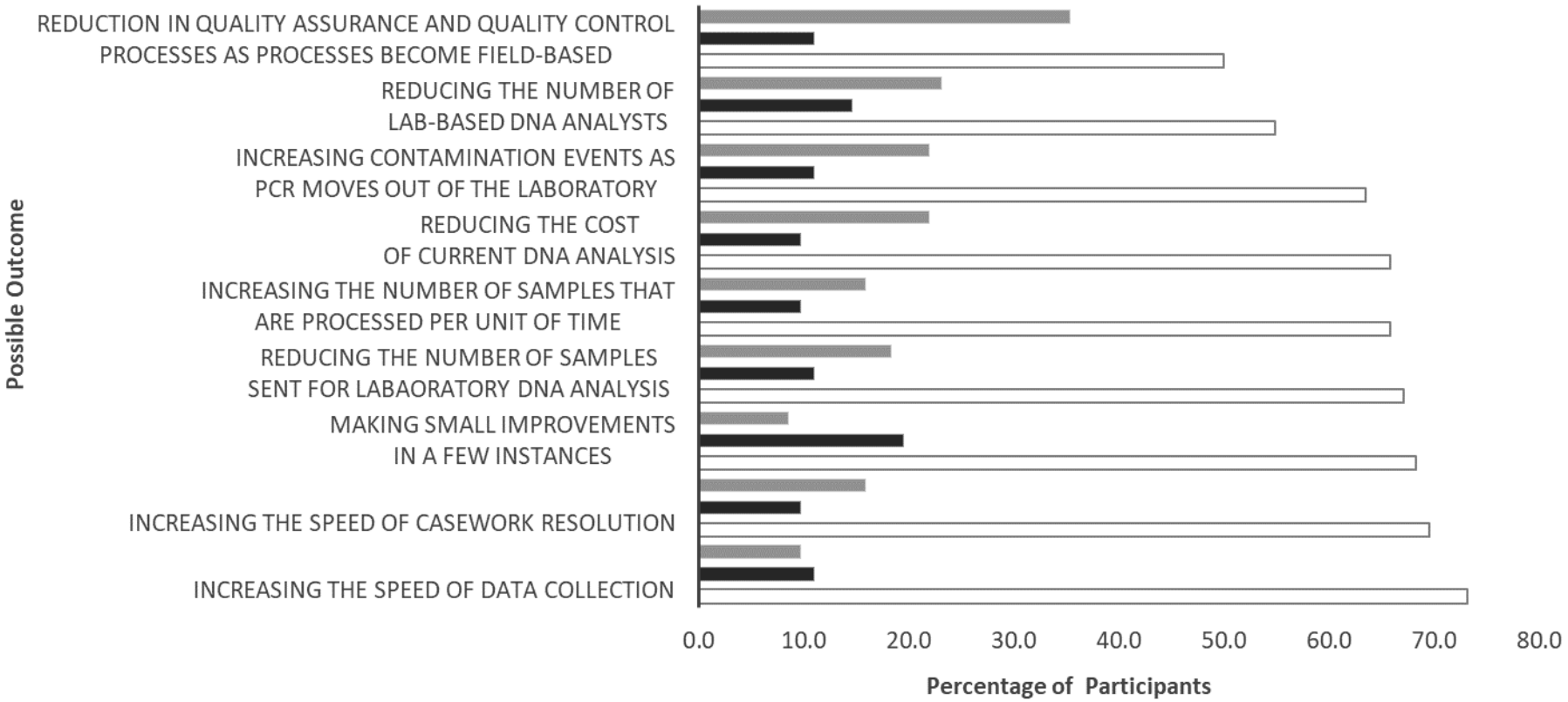

Figure 2. *Possible outcomes when adopting field-based DNA instrumentation in the field. Respondents answered either 'likely' (white bars); 'impartial' (black bars); or 'unlikely (grey bars) when asked about each of the possible outcomes. Number of responders to question was 30 for field-based practitioner, 22 for lab-based practitioner, 9 for desk-based individual, and 20 for lab-based researcher.*

190 *4.3. Perceived benefits regarding the adoption of field-based instrumentation*

191 Analysis shows that participants believe that once introduced, the impact of the field-based
192 intervention would be positive (Table 3). Ranking of possible outcomes by participants shows
193 that increasing the speed of data collection, increasing the speed of casework resolution, and
194 increasing the number of samples processed per unit of time were identified as 'likely'
195 outcomes (Figure 2). When asked 'how long should it take to prepare a sample for analysis?'
196 36% of participants selected ≤ 30 minutes; 21% selected ≤ 10 minutes; 40% selected ≤ 5
197 minutes; and 3% selected ≤ 1 minute. As such 97% of potential users would be satisfied if
198 sample preparation time was within 5 minutes. When asked 'how long should it take to generate
199 usable and understandable data?' 8% of participants selected ≤ three hours; 29% selected 60-
200 90 minutes; 36% identified 30-60 minutes; and 27% selected less than 30 minutes. As such the
201 data suggest that 73% of participants would be happy with a test that runs within 1 hour.

202 Typically, developers have increased the speed of current processes by integrating sample
203 purification (DNA extraction) and sample amplification (PCR) steps [19, 28, 29]. This has often
204 been in due to the high demands in law enforcement to analyse more DNA samples faster at
205 less expense to increase the speed of casework resolution [13, 30, 31]. One mechanism explored
206 in human forensic analysis is the idea of using field-based testing for sample triage at the crime
207 scene which can bring objectivity to evidential assessment and can reduce the number of
208 samples sent for analysis prior to obtaining a result [32]. Such benefits may also be translated
209 to practitioners of wildlife forensic casework which remains expensive due to the cost related
210 to the development of in-house protocols and the low sample throughput which raises the cost
211 of analysis per sample. A development target for commercial groups has been to perform DNA
212 analysis in under an hour from the point of sample collection. The data presented here supports
213 this as a developmental goal.

Another key developmental driver has been on ease-of-use. When asked 'what level of user expertise should field-based instrumentation be aimed at?' 28% of participants selected 'DNA aware CSI'; 22% selected 'Forensic Aware Enforcement Officer'; 22% selected 'anyone with 5 minutes training'; 16% selected 'Good DNA knowledge'; and 12% selected 'DNA expert' (Table 3). Interestingly, it was the desk-based and the field-based practitioner groups who selected 'DNA Expert' as an acceptable descriptor of an end user in contrast to the lab-based practitioners and lab-based researchers who did not select this descriptor at all. Overall, the data suggests that there is a clear expectation that the instrumentation should be aimed at non-laboratory-based individuals. Ease of use also relates to data interpretation. When asked 'what features of the analysis and software are required' 21% of participants selected 'graduated percentage confidence in the result' and 19% selected 'software-based interpretation'. Such functionality would make it extremely easy for field practitioners, especially as the percentage match result is already provided through existing sequence similarity searches. Interestingly, 14% of the participant's selected 'expert based interpretation' suggesting that there is a desire for some further verification of the result by another individual. Also, 12% selected 'binary yes/no answer'; 12% selected 'probabilistic result' and 11% selected 'raw accessible data'. Interestingly, only 10% selected 'weighted and phrased for use in forensic casework' which suggests that participants currently see little need for the analysis software to format the data ready for submission as evidence. This may be due to the existing reliance on forensic laboratory staff to present data in court and unwillingness by the community to automate the interpretation process. However, it should be noted that such automation has already been partially achieved with DNA data in the form of the STRmix^{TM} expert software [33] and validation guidelines exist to support software developers [34].

Table 3. *Participant groups response to impact of intervention, end-user expertise, and optimal location for deployment.*

Study Question	Response	Desk-based Individual	Lab-based Researcher	Lab-based Practitioner	Field-based Practitioner	Total Average (%)
		Percent (%)				
A) Impact of intervention	Positive Effect	80.0	85.0	86.4	87.0	85.6
	No Effect	0.0	15.0	9.1	6.5	8.4
	Negative Effect	20.0	0.0	4.5	6.5	6.0
B) Expertise descriptors for possible end-users	DNA Expert	25.0	0.0	0.0	22.2	11.8
	Good DNA Knowledge	8.3	27.8	21.4	7.4	16.2
	DNA Aware CSI	16.7	33.3	28.6	33.3	28.0
	Forensic Aware Enforcement	16.7	16.7	28.6	25.9	22.0
	Anyone	33.3	22.2	21.4	11.2	22.0
		Weighted Percent (%)				
C) Location for field-based deployment	Offices	0.0	10.5	20.0	13.3	13.0
	Customs and Border Stations	38.5	39.5	37.1	28.9	35.1
	Vehicles	7.7	18.4	17.1	17.8	16.8
	Field Sheltered	53.8	18.4	22.9	24.4	25.2
	Field Unsheltered	0.0	13.2	2.9	15.6	9.9
D) Features of analysis and interpretation desired	Software based interpretation	18.5	16.0	20.0	22.2	19.3
	Graduated % confidence in result	22.2	16.0	26.7	20.4	20.5
	Expert based interpretation	18.5	12.0	13.3	14.8	14.3
	Binary Yes/No Answer	7.4	12.0	10.0	16.7	12.4
	Probabilistic	7.4	14.0	13.3	13.0	12.4
	Raw data accessible	11.1	16.0	10.0	7.4	11.2
	Weighted and phrased for use in casework	14.8	14.0	6.7	5.6	9.9

Results are the percentage based on the number of responders. Number of responders to question A) was 31 for field-based practitioner, 22 for lab-based practitioner, 10 for desk-based individual, 20 for lab-based researcher. Number of responders to question B) was 27 for field-based practitioner, 14 for lab-based practitioner, 12 for desk-based individual, 18 for lab-based researcher. Number of responders to question C) was 27 for field-based practitioner, 14 for lab-based practitioner, 12 for desk-based individual, 18 for lab-based researcher. Number of responders to question D) was 26 for field-based practitioner, 14 for lab-based practitioner, 12 for desk-based individual, 18 for lab-based researcher.

With regards to the most suitable location for field-based testing, 35% of participant's selected 'customs and boarder stations'; 25% selected 'field sheltered'; 17% selected 'vehicles'; 13% selected 'offices'; and 10% selected 'field unsheltered'. This represents a possible division in relation to what an instrument is expected to do. It is likely that customs, border posts and offices have electric power supplies which would allow the use of any instrumentation that requires power, including larger desk-based instrumentation. Field stations may require a generator or require battery powered instrumentation or utilise methods that require no power source for analysis such as lateral flow and immunoassay-based devices.

When polled on 'how many samples would be run each week using field-based instrumentation' 70% of total participants stated they would analyse at least five samples a week (Figure 3) with the greatest usage identified in the field-based practitioner group. Usage was identified in other groups also, although it is difficult to assess whether this represents a true need or whether participants were responding from the point of view of a field practitioner. It is likely that usage will vary between different applications and jurisdictions so further insight may be required as specific species of interest and enforcement groups are identified who may become early adopters of field-based analysis.

The data reveals that respondents broadly favour the adoption of field-based, office-based or non-laboratory-based instrumentation. Furthermore, there is support for deployment at borders and ports suggesting that detection of trafficked items is the preferred application. With regard to data interpretation (Table 3) the two most common requests, representing almost 40% of respondents, was for 'software based interpretation' with a 'graduated % confidence in the result', directly relating to accuracy or percentage similarity akin to DNA sequence similarity searching [35]. This would suggest that the greatest proportion of individuals would like minimal hands on data analysis with fewer individuals wanting access to the raw data.

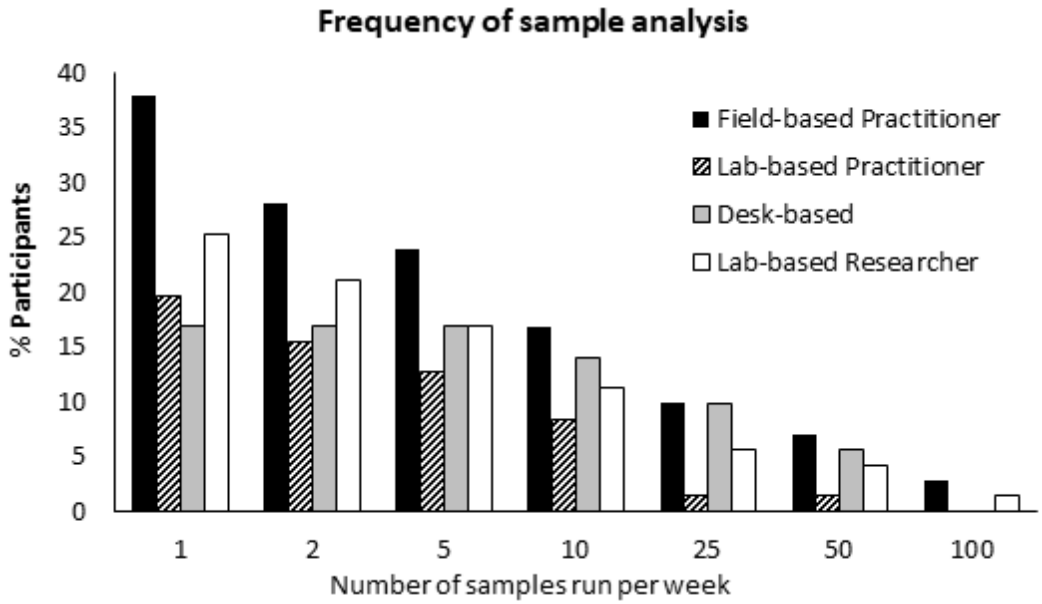

Figure 3*. Cumulative total showing the number of participants (%) that would run at least 1, 2, 5, 10, 25, 50 or 100 samples per week using field-based DNA analysis. Number of responders to question was 27 for field-based practitioner, 14 for lab-based practitioner, 12 for desk-based individual, and 18 for lab-based researcher.*

4.4. Investigative Questions, Species and Sample Types

Beyond legal casework to directly support prosecutions, wildlife forensic science includes a range of stakeholders working in areas of academic research, trade monitoring, supply chain verification and intelligence gathering. The development of a single solution to field-based testing is therefore complicated by the different species, objectives and priorities in play. Our results show that determining species identity is currently the most common form of analysis performed (Figure 4). For this type of analysis forensic providers match the DNA sequence of the unknown sample to 'known' DNA sequences stored on open-access databases [36, 37]. However, even this common approach suffers from limitations as the databases are unregulated, leading to uncertainty in the result, and are sometimes not populated with data from the species of interest. To combat this problem, the wildlife forensic community are developing the ForCyt DNA database [38], a fully-regulated database of species that are commonly encountered in forensic investigations. Such a database would make the development of a field-test more

achievable, but may still require different design strategies depending on whether the question is one of identification (what species is it?) or detection (is it Tiger?). Typically, molecular tests are developed to detect a specific analyte, addressing the closed form of the question [i.e. 39-42]. When an open identification question is asked, the emphasis shifts toward building a test capable of identifying every single species of interest and consequently becomes more difficult. The preference to ask open questions often severely limits what a laboratory can do and investigators are often asked to be more specific with their request.

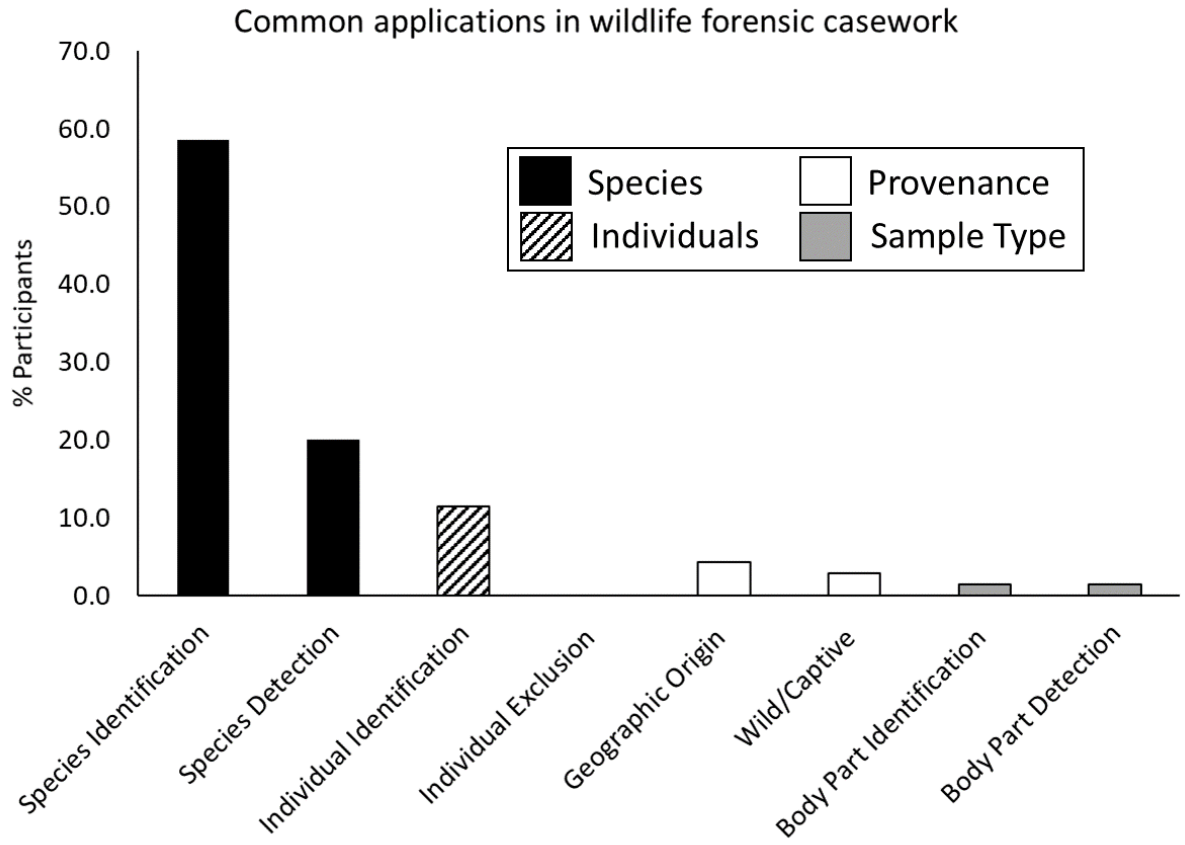

Figure 4. *Common applications in wildlife forensic casework. Number of responders to question was 25 for field-based practitioner, 20 for lab-based practitioner, 8 for desk-based individual, and 19 for lab-based researcher.*

Analysis pertaining to individual identification and determination of geographic origin or wild/captive assessment are less commonly required because the tests are expensive to develop,

297 niche in application and often require de-novo collection of appropriate population reference
298 databases [43-45]. The least common question is to identify whether a sample belongs to a
299 specific part of an animal. Given that determining species identity through open or closed
300 questions is required in the majority of instances, it is seems sensible that industry groups
301 develop approaches that seek to address this type of question.

302 When asked to identify 'which group of fauna/flora is most often encountered', 63% of
303 participant's selected 'mammal'; 13% selected 'birds'; 9% selected 'fish'; 7% selected
304 'invertebrates'; 4% selected 'timber' 1% selected 'reptiles'; and 4% selected 'various'. With
305 regards to the 'type' of sample commonly encountered, 35% of participants selected 'meat/body
306 parts/organs'; 21% selected 'bones/teeth/scales'; 16% selected 'live animal'; 15% selected
307 'skins/pelts/furs/wools'; 14% selected 'liquid mixtures'; 14% selected 'whole dead animals';
308 11% selected 'powdered derivatives'; 10% selected 'horns/ivory'; and 7% selected
309 'pods/seeds'. The range of sample types highlights a problem for developers of field-based
310 molecular approaches for wildlife forensic applications. Developing a detection platform that
311 works across an entire range of samples types is difficult, and in some instances has limited the
312 use of non-lab based systems to a single form of analysis, such as individual identification, on
313 a single sample type, such as buccal swabs [e.g. 15]. Other systems have also recommended
314 additional expertise and time spent on pre-processing steps [e.g. 46] to allow complete analysis.
315 Indeed, the challenging samples encountered by forensic scientists continue to be the focus of
316 development for laboratory processing, let alone field-based applications [47].

317 To further understand taxon importance, participants were asked to rank a list of flora and fauna
318 (pre-identified by the authors as forensically relevant) and thereby identify which is most likely
319 to benefit from a field-based detection system. The weighted data shows the top five groups
320 identified are African rhinos, African elephant, Asian elephant, tiger and pangolin (Figure 5).
321 The identification of four flagship taxa and pangolin requires some discussion. The main

forensically relevant samples collected from elephants is ivory, which is often unprocessed and exported to Asia where it is in high demand, especially in China [48]. While it can be readily identified morphologically, ivory from African and Asian elephants can be mixed making it difficult to distinguish between the two groups without laboratory testing. Forgers are also becoming increasingly adept at creating fake ivory pieces from bone, teeth, horn, plastic and resin which are sold as real ivory [49, 50]. Furthermore, the use of population assignment approaches has been used to identify poaching hotspots [51] suggesting that population assignment may be the primary application when considering field-based molecular approaches for ivory. Pangolin scales can also be identified morphologically, although not to sub-species level. As with elephants, the broad distributions of certain pangolin species from both Asia and Africa, may suggest that the primary application of any test is to differentiate between geographic regions to support investigations and identify poaching hotspots. It should be noted that any test capable of population assignment in these species will also simultaneously perform species identification which remains an important consideration. Forensically relevant samples of the two remaining top-ranked species can include tiger bone and rhino horn, both of which can be ground up into powders for inclusion in Traditional Asian Medicines (TAM) [52]. The lack of any identifying characteristics when handling these processed samples suggests that a simple field-test for species identification would support investigations that involve the analysis of TAM products.

The inclusion of commercial fish species as the sixth most likely to benefit from field-based testing suggests that the illegal fishing, landing and species substitution of high value species with low value species also represents a clear development goal as species detection and verification is something that is required throughout the food chain [53, 54]. To understand and develop an assay for commercial fish species further research is needed with regard to species prioritisation. Research has listed demersal fish, salmon, trout and smelt as having the highest

levels of illegal fishing [55] but it remains difficult to identify a single species to target with 54% of the stock/species categorised as being at high risk of illegal, unregulated and unreported fishing [56]. Indeed, it is likely that the development of field-based testing in fisheries and food supply chains will be prioritised over methods developed for critically endangered species, as fish identification represents a larger end-user market and has an immediate relevance to human health and food safety. It is also considered likely that the development of a system that works on fish species will be easier to apply, given the samples commonly encountered include single source, DNA rich, tissue and muscle.

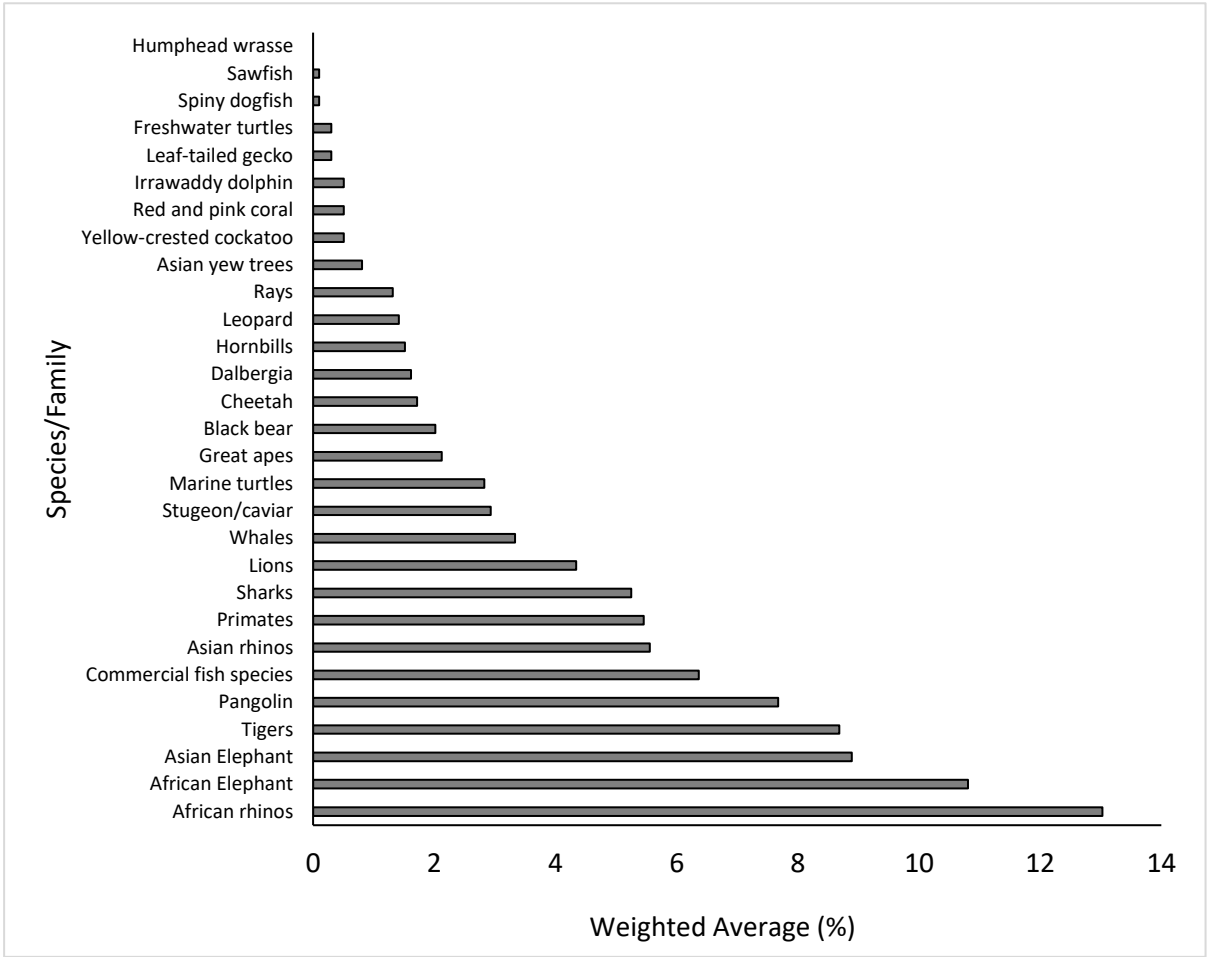

Figure 5. *Ranked species in order of most likely to benefit from a field-based DNA profiling system. Each participant was asked to rank what they thought were the top 5 species. Results were ranked using a weighted average giving more importance to the species selected first. Number of responders to question was 21 for field-based practitioner, 21 for lab-based practitioner, 8 for desk-based individual, and 20 for lab-based researcher.*

5. Summary

This questionnaire has identified a need for non-laboratory detection applications in wildlife forensic science. The results highlight a series of end user expectations and concerns that industry groups and developers can address either through mapping requirements to existing systems or developing entirely bespoke assays or instruments. The key elements identified are broadly in alignment with the expectations placed on human-based detection platforms:

- **Results within one hour from the start of sample processing**
- **Easy to use tool with simplified data interpretation**
- **95% accuracy of identification**

At this moment in time there are a number of systems that are close to fulfilling some of the requirements outlined by this research but no assay or instrument currently fulfils all the requirements. Instruments of note include, the Oxford Nanopore Technologies MinION [57-59], a highly portable system that meets cost requirements and can be used in the generation of data suitable for species identification. It meets limit of detection requirements, but the current end-users require a high level of experience at sample preparation and result interpretation although simple disposable consumables and software are under development to address these limitations. The ParaDNA system [60, 61] has shown potential as a forensic screening system and has been developed specifically for end users with no laboratory experience. Data interpretation is by automated software which requires no expertise to interpret. Accuracy is high but the system is only within the budget of a small portion of the participants of this questionnaire. Furthermore, it only runs pre-developed assays which may reduce the likelihood that a wildlife assay can be used in conjunction with the system without collaboration from the industry developers. Immunoassays [62-64] are low cost, easy to use and suitable for field and indoor conditions. However, issues exist regarding sensitivity and specificity and they do not

always work with degraded samples. Typically, molecular detection tests with low cost show questionable accuracy. However, it is important to recognise that such tests have an important function in forensic casework as presumptive tests.

Both presumptive and diagnostic tests have utility in an investigative framework but there is yet to be a test that combines low cost with high accuracy. Further research looking at mechanisms to achieve this are ongoing [65, 66] but are likely 5-10 years away from being commonly used. As such, if non-laboratory-based detection systems are to be utilised in the interim period it is likely to be done on an ad-hoc basis with each end user group identifying the system that specifically suits their needs and collaborating with industry developers to understand ways in which it can improved to better suit their purpose. A likely stepping stone towards true field-based tools is the early adoption of some of these technologies within forensic laboratories in low and middle income countries which currently lack relatively expensive genetic analysis instrumentation and are the sources of many of the species involved in the illegal wildlife trade. Adoption of cheaper and faster tests will significantly enhance regional enforcement action by initially building capacity within such dedicated facilities whilst the developments required for deployment outside of a laboratory are validated. Finally, it is essential that community groups help develop a series of guidelines for the field-based validation of detection systems that can be readily used by enforcement groups and non-laboratory trained individuals. In doing so, many of the concerns identified during this study will addressed in preparation for the widespread adoption of future field-based analysis systems.

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612 **Supplementary Material 1**

Q1	Tick which of the following roles most closely matches your current position		Tick
	A	Lab-based, forensic case worker	
	B	Field-based, wildlife crime investigator	
	C	Lab scientist - other (food standards, conservation etc)	
	D	R&D Project Manager (Academic/Industry)	
	E	Customs/Boarder Control	
	F	Police/Enforcement Officer	
	G	Charity/NGO/Policy Representative	
	H	University researcher	
	I	Other (please state)	

Q2	Of the wildlife samples you work with, what percentage requires some form of laboratory based DNA analysis?		Tick
	A	None	
	B	<20%	
	C	20-40%	
	D	40-60%	
	E	60-80%	
	F	100%	

Q3	How familiar are you with current, field-based, DNA instruments?		Tick
	A	Very familiar with current technology and approaches	
	B	Familiar with some platforms	
	C	Some literature based knowledge	
	D	Very little known	
	E	Not previously aware of field-based DNA instrumentation	

Q4	Rank each of the following issues (1-8) regarding how they prevent the use of current field-based instrumentation in wildlife		Tick
	Note: You can't rank them equally and you have to use all values 1-8 in your selection.		
	A	Cost	
	B	The colour of the instrumentation	
	C	Ease of use	
	D	Lack of funding for purchasing	
	E	Accuracy of the test and instrument	
	F	Sensitivity of the test and instrument	
	G	Lack of an instrument that suites my needs	
	H	Lack of an assay that I can use	

Q5	How helpful would field-based DNA instrumentation be in your current work?		Tick
	A	Extremely Useful	
	B	Useful	
	C	Slightly useful	
	D	No effect	
	E	Slightly unhelpful	
	F	Unhelpful	
	G	Extremely unhelpful	

Q6	Score each of the following possible outcomes of adopting field-based DNA instrumentation in the field from 1 -7 1= Extremely likely, 2= Very likely, 3= Likely, 4= Neither likely or unlikely, 5= Unlikely, 6= Very unlikely, 7= Extremely unlikely		Tick
	A	Reducing the cost of current DNA analysis	
	B	Reducing the number of samples sent for laboratory DNA analysis	
	C	Increasing the speed of data collection	
	D	Increasing the speed of casework resolution	
	E	Increasing the number of samples that are processed per unit of time	
	F	Reducing the number of lab-based DNA analysts	
	G	Making small improvements in a few instances	
	H	Reduction in quality assurance and quality control processes as processes become field-based	
	I	Increasing contamination events as PCR moves out of the laboratory	

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Q7	Tick the wildlife group that you most commonly encounter in your role		Tick
	A	Reptiles	
	B	Mammals	
	C	Birds	
	D	Fish	
	E	Invertebrates	
	F	Amphibians	
	G	Timber	

Q8	Assign a percentage score (0-100%) to each of the following sample descriptions based on how often you come across these		Tick
	A	powdered derivatives	
	B	Live animals	
	C	meat/body parts/organs	
	D	whole dead animals	
	E	pods/seeds	
	F	skins/pelts/furs/wools	
	G	horns/ivory	
	H	liquid mixtures	
	I	bones/teeth/scales	

Q9	Rank the following forensic casework questions (1 most common - 8 least common) in terms of which is the most often asked in		Tick
	A	What species is it?	
	B	Is it species XXXX?	
	C	Can you identify the individual animal who left the sample using a DNA database or match probability calculation?	
	D	Can you exclude individual XXXX as the animal who left the sample?	
	E	Where did the animal come from?	
	F	Did the animal come from the wild?	
	G	What part of the species does the sample come from?	
	H	Does the sample come from the XXXX part of the animal?	

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Q10	Rank the following species (1-5) in order of most likely to benefit from a field based DNA profiling system					
	Asian Elephant		Primates		Hump head Wrasse	
	African Elephant		Pangolin		Sawfish	
	Asian rhinos		Leaf-tailed Gecko		Red and pink coral	
	African Rhinos		Hornbills		Spiny dogfish	
	Lions		Yellow-Crested Cockatoo		Sturgeon/caviar	
	Tigers		Whales		Commercial Fish Species	
	Leopard		Irrawaddy Dolphin		Asian Yew Trees	
	Cheetah		Freshwater turtles		Dalbergia	
	Black bear		Marine Turtles			
	Great Apes		Sharks			

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Q11	If you had to select a single species to prioritise developing a field based DNA assay for, what species would it be and why?
	Answer

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Q12	What level of user expertise should field-based DNA instrumentation be aimed at?		Tick
	A	DNA Expert	
	B	Good knowledge of DNA approaches	
	C	DNA aware Forensic Investigators	
	D	Forensic Aware Enforcement Officers	
	E	Anyone with 5 minutes training	

Q13	Where do you see field-based instrumentation being deployed?		Tick
	A	Offices	
	B	Customs and border stations	
	C	Vehicles	
	D	Field sheltered	
	E	Field unsheltered	

Q14	How long should it take to prepare a sample for analysis on field based instrumentation?		Tick
	A	1 minute	
	B	5 minutes	
	C	10 minutes	
	D	Within 30 minutes	

Q15	What sort of samples should the instrument and test work on?		Tick
	A	Blood	
	B	Powdered derivatives	
	C	meat/body parts/organs	
	D	horns/ivory	
	E	liquid mixtures	
	F	bones/teeth/scales	
	G	Degraded samples	
	H	Samples mixed with environmental contaminants (e.g. soil/fauna)	

Q16	How long should it take to generate useable and understandable data from the time you collect the sample?		Tick
	A	<30 minutes	
	B	30-60 minutes	
	C	60-90 minutes	
	D	3 hrs	

Q17	How accurate does the test need to be?		Tick
	A	80% Accurate	
	B	85% Accurate	
	C	90% Accurate	
	D	95% Accurate	
	E	99% Accurate	
	F	100% Accurate	

Q18	How sensitive does the test need to be (how much biological material does it need to detect)? NOTE: Most Current laboratory DNA tests can routinely detect between 10 and 100 cellular copies of nuclear DNA, less if mtDNA is being used		Tick
	A	Single cell or 6.6pg DNA	
	B	10 cells or 66pg DNA	
	C	100 cells or 660pg DNA	
	D	500 cells or 3.3ng DNA	
	E	1000 cells or 6.6ng DNA	

Q19	What features of the analysis and interpretation are required?		Tick
	A	Software based interpretation	
	B	Expert based interpretation	
	C	Binary Yes/No Answer	
	D	Graduated % confidence in result	
	E	Probabilistic	
	F	Raw data accessible	
	G	Appropriately weighted and phrased for use in forensic casework	

Q20	What is the maximum you would pay for a single field-based DNA instrument?		Tick
	A	£100	
	B	£1,000	
	C	£5,000	
	D	£10,000	
	E	£50,000	
	F	£100,000	

Q21	Of the wildlife samples you work with, what percentage would you consider using field-based DNA analysis methods on?		Tick
	A	None	
	B	<20%	
	C	20-40%	
	D	40-60%	
	E	60-80%	
	F	100%	

Q22	What is the maximum you would pay for a set of reagents to perform your wildlife test		Tick
	A	£1 Per Sample	
	B	£10 Per Sample	
	C	£20 Per Sample	
	D	£50 Per sample	
	E	£100 Per sample	
	F	£200 Per Sample	

Q23	How likely are you to buy a field based DNA instrument if it performed according to your requirements and was within your budget?		Tick
	A	Very Likely	
	B	Likely	
	C	Unlikely	
	D	Very unlikely	

Q24	How many samples would you run per week?		Tick
	A	1	
	B	2	
	C	5	
	D	10	
	E	25	
	F	50	
	G	100	

Q25	How would you secure funds to purchase field-based DNA instrumentation		Tick
	A	Government Grants	
	B	Research Funding Bodies	
	C	Internal Institutional Based Funding Calls	
	D	NGO/Charity Funding	