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Is anti-doping analysis so far from clinical, legal or forensic targets?: The added value of close relationships between related disciplines

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There are many areas of common interest between anti-doping laboratories and those working in the clinical, legal and forensic fields. In addition to methodological similarities, there are aspects of the findings in sport drug testing that overlap with other fields in such a way that sport drug testing and clinical, legal or forensic work may benefit from mutual interaction. Three recent examples are presented from the author's experience. Case report 1 concerns the clinical relevance of hCG findings in sport drug testing as potential indicators of the presence of a (testicular) tumour in athletes. Case report 2 refers to difficulties that accredited laboratories can encounter due to differences between national legal systems and the administrative regulation systems of sport authorities. The example involves a network of blood collection for further autologous transfusion. Case report 3 relates to additional forensic-type investigations needed to interpret a situation where intoxication of a whole delegation was responsible for apparent doping cases. Clinical, legal and forensic fields must recognize the added value that some results and developments coming from anti-doping laboratories may have. At the same time anti-doping analysts should be aware of new issues, methodologies and problems appearing in related fields.

Keywords: co-operation; related disciplines; clinical diagnostics; tumour marker; blood transfusion network; overlapping jurisdictions; deliberate team intoxication; sample tempering; publications

Introduction

Doping control analysis aims to detect the use of a doping agent by an athlete. The consequences, when this is confirmed, usually involve the imposition of a sanction. Given the effect of such a penalty on a sportsperson's life, the standards of quality, custody of samples, the use of appropriate methodology and the accuracy of results that are required put anti-doping control tests among the most reliable analytical tests using biological samples.

The World Antidoping Agency[1] has strict rules and regulations, the goal of which is to guarantee that anti-doping laboratories have the proper accreditation to perform their tasks. The strict standards demanded of anti-doping laboratories equal or exceed many of the usual standards for legal or forensic ones. Moreover, some of the target analytes of sports drug testing are metabolites and hormones similar to those produced naturally by the body, which confers some clinical relevance to the anti-doping analyses.

However, apart from methodological similarities, there are sometimes aspects of the findings in sport drug testing that partially overlap with aspects of other areas of work in such a way that both the sport drug testing and those other fields may benefit from mutual interaction. It is not surprising, then, that anti-doping scientists are often present at, and make important contributions in, forums and meetings of societies belonging to those other fields (see Table 1) and in multidisciplinary publications.[2–4]

Scientists working in sports drug testing have often experienced such an overlap with neighbouring areas. In this paper some recent examples from the author's experience are presented that illustrate such enriching possibilities.

Case Reports

Case report 1: clinical (tumour marker)

Subject 1

A sportsman tested positive for human chorionic gonadotrophin (hCG) in two samples with a time span of 3 months in two different laboratories, one of them being the laboratory directed by the author, with a negative sample in between. Knowing that hCG or metabolites could be a marker for a testicular or similar tumour,[5] the Sport Authority was advised to consider the possibility of a clinical explanation as an alternative to doping. The sportsman was informed and subjected himself to medical investigation, which resulted in no clinical explanation for the findings. The Federation Doping Panel and the Court of Arbitration for Sport imposed a sanction.

Five years later another sample was found to be positive for hCG in our laboratory. It was eventually revealed to be from the same sportsman. This time the laboratory strongly urged an additional medical examination before taking a decision. The result was that a tumour (seminoma) was diagnosed, which required orchidectomy. Some of the questions raised were: 'Did the swimmer have the tumour 5 years earlier?' 'Was the endocrinological investigation done properly at that time?' 'Was it too early, at that time, for

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Table 1. Some examples of recent meetings where public presentation on non-analytical aspects of doping control have been requested from the author

Clinical and science

- 18th IFCC-EFCC European Congress of Clinical Chemistry and Laboratory Medicine, Innsbruck
- ICSEMIS International Convention on Science, Education and Medicine in Sport, Guangzhou
- European Science Open Forum, Barcelona
- 10th European Congress of Endocrinology, Berlin
- Congreso Paulista de Farmaceuticos, Sao Paulo
- XXX FIMS Worlds Congress of Sports Medicine, Barcelona
- European Seminar on Neuroimaging and Addiction, Barcelona
- XXV Congreso Sociedad Española de Bioquímica Clínica y Patología Molecular, Bilbao
- 2 Congresso Nazionale Reumatologia e Sport, Cagliari

Legal and forensic

- V Latin American Regional Meeting of the International Association of Forensic Toxicologists TIAFT Porto Alegre
- Prosecutors updating programme, Ministry of Justice, Madrid
- Master in Forensic Toxicology, Universidad del País Vasco, Bilbao
- Joint Meeting of the Gesellschaft für Toxikologische und Forensische Chemie (GTFCh) and The International Association of Therapeutic Drug Monitoring and Clinical Toxicology (IATDMCT), Munich
- Course on Law and Sport, Ministry of Justice, Madrid
- First International Congress on Law in Sport, Barcelona
- Advances in Forensic Toxicology, University of Madrid
- 19th Congress IALM International Academy of Legal Medicine, Milano

physical signs of a tumour?' 'How should rising and falling hCG urinary concentrations be interpreted?'

Subject 2

A sportsman was found positive for hCG by one method out of two in another laboratory. It was known that the two methods had different cross-reactivity to hCG fragments (Figure 1). Priority was given by the Federation to the confidential clinical follow up before it considered imposing sanctions. The medical team was informed by the Doping Review Board of the federation involved and was requested to investigate. It was told: 'The athlete is to be advised that this analytical finding may indicate the presence of a medical problem for which prompt investigation and treatment is advised. The athlete is to be advised that the details of the results of medical investigations should be forwarded, in confidence to. . .' However the results did not indicate any clinical abnormality.

One year later, a positive hCG result (by two different immunological assays) was detected in our laboratory. We reported that 'Some clinical situations may be compatible with the adverse finding obtained. A medical examination is recommended when considering the case.' Eventually it emerged that this sample was from the same sportsman. This time, again, the international federation concerned indicated that 'the athlete and national federation officials be advised in the strongest terms, to seek specialized medical care in order that an appropriate investigation of these findings might be carried out.'

This time the clinical examination reported the finding of a testicular cyst, the sportsman being referred to a specialist in hCG interpretation. Considerations of confidentiality prevented the release of further information on the case.

Case report 2: legal (blood transfusion network)

As a result of suspicions, police raided addresses in several cities and laboratory equipment was found that was used for blood transfusions, together with more than 200 bags of blood (plasma and/or whole blood/red blood cell concentrates) and large quantities of doping agents. The raids were based on the law prevailing at the time in the country: doping was not included but violation of public health issues was considered. Initially the judge asked our laboratory to search for the presence of prohibited substances in the plasma samples. Complete screening was carried out without finding any relevant doping substances out of eight erythropoietin (EPO) concentrations higher than normal. The extent to which those concentrations corresponded to recombinant human EPO (rhEPO) had to be investigated. However, the methodology existing at that time for rhEPO had been developed for urine[6–7] and was not applicable to blood. Accordingly, in order to cover the list of prohibited substances, an adaptation of the method for urine rhEPO needed to be developed in house that could be used for blood samples. The methodology, based on introducing a microplate immunopurification step, was developed and later published,[8] and the results confirmed that those eight suspicious samples actually contained rhEPO.

The judge considered that the presence of substances such as rhEPO did not constitute a serious danger to the health of the athletes involved and, based on a non-doping expert report, came to the conclusion that the events could not be considered to be criminal according to the law in effect at the time.

The judge's decision did not take into account the inappropriate system of labelling the blood bags, which could become very risky if an incorrect reinfusion were to be created by mislabelling. Subsequently, different national and international sport bodies have unsuccessfully fought to overturn the denial of authorization of using those elements for sanctioning by the sport authorities. Only those countries where the law authorizes the prosecution of procurement of doping as a criminal activity were allowed to use blood samples for DNA identification (see Figure 2). Among them, a preliminary order to supply blood to one of the foreign prosecutors for DNA fingerprinting was later reversed by the judge on the basis of lack of jurisdiction but after blood had already been transferred. The extent to which the disciplinary actions raised afterwards by the foreign prosecutor against a sportsman based on the DNA analysis will prevail are to be defined by the Court of Arbitration for Sport.

During all the cases, our laboratory was only authorized to act under the law of the country applicable at the time of the raids without being able to transfer the results to sports authorities for further evaluation and sanctioning. Just four months after the raid, a law that would have allowed the prosecution of instigators of doping and probably an administrative sanction on the athletes involved was approved by the national Parliament.

Case report 3: forensic (deliberate team intoxication)

During a pre-Olympic tournament for qualification for the 2008 Olympics (only the winning team qualified for the Games) a female national team complained of illness in the hotel the day before the semi-final match, initial symptoms being defined as a vertiginous

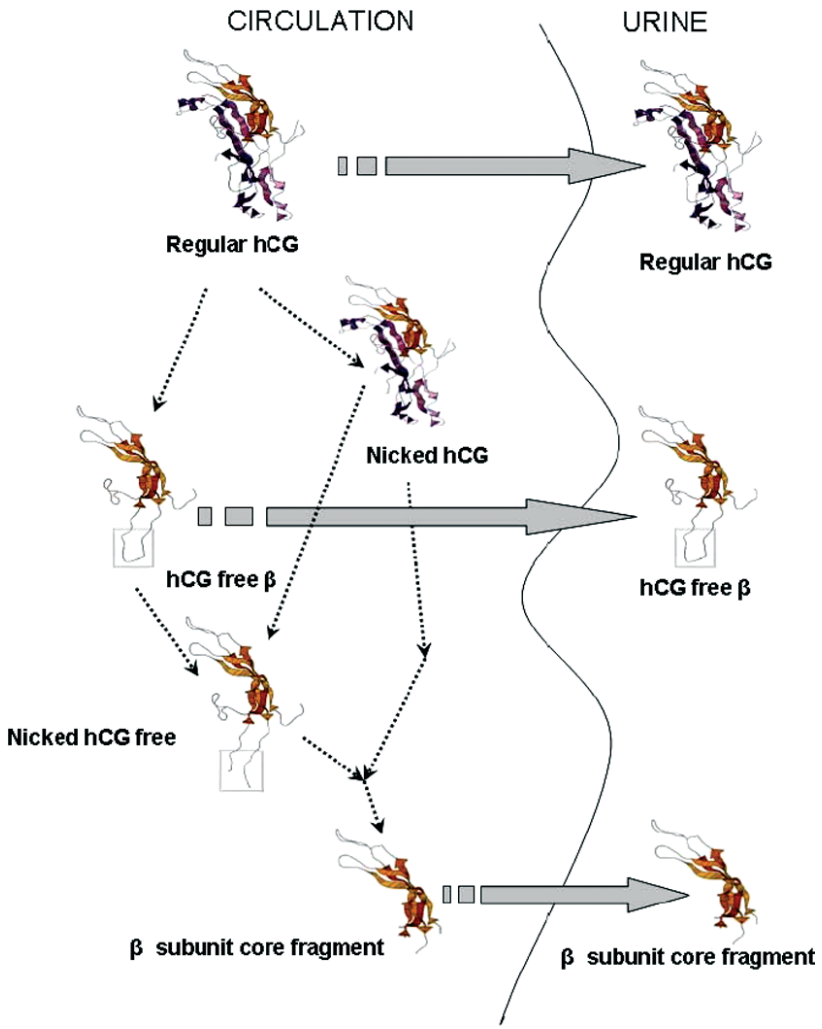

Figure 1. Refer to case report 1: simplified scheme of the disposition of hCG in the human body. Adapted from reference[17].

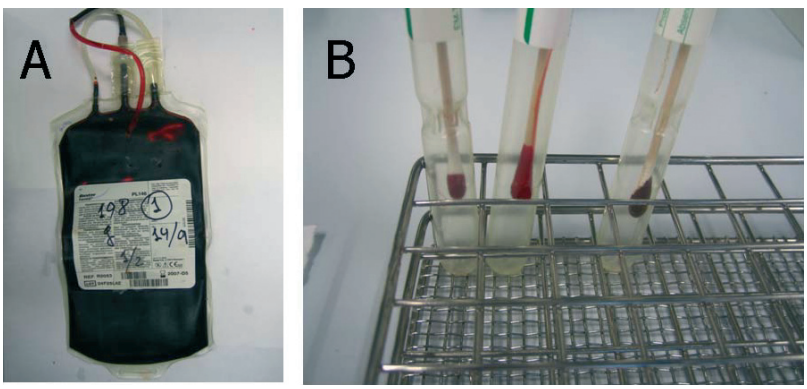

Figure 2. Refer to case report 2 – bag of blood confiscated and samples from it for DNA analysis.

sensation, weakness of legs, dizziness and shivering. Longer lasting symptoms were general weakness, fatigue and a feeling of being dazed. The causal agent was apparently linked to some exposure during the dinner that day. The delegation filed a corresponding medical record about the incident and also complained that players were unable to sleep after being bombarded by telephone calls in their hotel rooms. Others felt ill after drinking water given to them during matches. The team eventually won the semi-final match and also the final one against the team of the organizing country and qualified for the Olympics.

Once back from the tournament, two members of the team (subject A and subject B) tested positive for ecstasy (metylenedioxymethamphetamine, MDMA). Given precedents of irregularities during the tournament, all members of the delegation were asked to deliver samples of hair, blood and urine for further investigation. A logical question was: could the illness of the

majority of the members of the whole delegation have been caused by group intoxication by MDMA? Hair analysis had the answer: all subjects who had suffered symptoms of intoxication appeared to have some amount of MDMA in the segments corresponding to the time period of the tournament. This fact included even the president of the national federation and his wife, both more than 70 years old. Duplicate blind-coded hair samples were also sent to an independent laboratory in Germany for another analysis. They confirmed the finding that 11 out of 14 subjects showed traces of MDMA in the hair segment corresponding to the time of the event but none in the others.

One of the positive doping cases (subject A) could be explained by intoxication at the previous dinner. However, the other positive case (subject B), which corresponded to one of the few players without signs of intoxication or MDMA in the hair, was found positive some days after the dinner, at the final match. This time, the enormous amount of MDMA detected (Figure 3) suggested a potential manipulation of the sample as it corresponded to a dose described as being lethal in some forensic reference books[9] or publications.[10] The rest of the competitors' urine sample, which had been kept in the original anti-doping laboratory, and another authenticated urine sample from the same player collected by a notary, were sent to another laboratory in Germany for verification of the steroid profile.[11–12] Differences observed (Table 2) indicated that both samples belonged to different subjects and that some kind of manipulation had been introduced in the tournament's sample.

With all these data, the juridical commission of the International Federation called a hearing, where eventually it was resolved that the first player (subject A) had committed a rule violation (it was MDMA in her body during the semi-final match) but she bore no fault or negligence and accordingly the period of ineligibility in respect of that violation was eliminated. As regards the second competitor (subject B), she had not committed any anti-doping violation as the tested sample was not her urine.

Discussion

The interaction between doping testing and other related areas is clear. Regarding impact on health, the association between doping agents and undesirable side effects has always been evident.[13–17] Taking into account that some of the doping substances recently introduced in the WADA prohibited list are hormones and peptides similar to natural ones, the overlap between doping control and clinical fields is to be expected. With the increasing trend for doping analysis to incorporate indirect biomarkers (resulting from the physiological action of banned substances), which are affected by different disease states of the individual, the relevance of doping results for health related issues will increase.

The cases reported in this paper illustrate different aspects of the multidisciplinary nature of anti-doping analysis. The two subjects in case report 1, having had hCG findings that were eventually shown to have come from a tumour, illustrate how biological marker-related analysis may have some diagnostic possibilities that must not be disregarded. In particular, hCG is a molecule with interpretation difficulties, as illustrated by previous reports.[5,18–20]

Non-throphoblastic tumours produce relatively low increases of hCG,[5] with concentrations closer to potential doping cases. The nature of the hCG fragment(s) (Figure 1) responsible for the

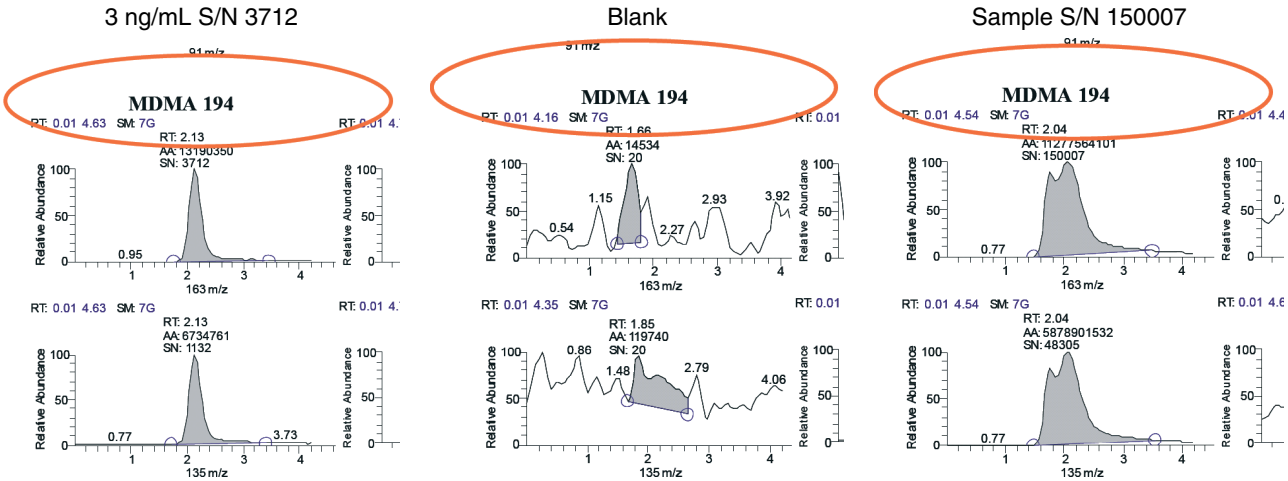

Figure 3. Refer to case report 3. HPLC-ESI-MS/MS screening analysis for a reference sample (left), a blank sample (middle) and the original A sample (right) collected from Subject 2. Data received from Dr G. Rodchenkov (Moscow).

Table 2. Refer to case report 3. Steroid profile parameters of the original sample collected at the final match for subject 2 compared with that of one authenticated sample from the same player. Data received from Dr H. Geyer (Cologne)

Sample	AND (ng/mL)	ETIO (ng/mL)	ADIOL (ng/mL)	BDIOL (ng/mL)	T (ng/mL)	E (ng/mL)	T/E
Original A	4538	4801	35,8	114,7	13,8	34,0	0,41
Original B	4510	4804	34,4	113,6	14,3	34,5	0,41
Authenticated	2190	2990	14,9	21,3	$<0,3$	7,7	$<0,04$

AND: androsterone. ETIO: etiocholanolone. ADIOL: 5α-androstane-3α,17β-diol. BDIOL: 5β-androstane-3α,17β-diol. T: testosterone; E: epitestosterone

immunoreactivity in common assays may also give a clue to the origin of the compound because it is known that in the urine of young, non-doping subjects the β subunit core fragment predominates as compared with the hCG free β and regular hCG.[21] However the lack of immunotests specifically addressing hCG forms in urine complicates the interpretation. In this context, anti-doping scientists should be aware of this situation in order not to overlook clinically important results (even if they are irrelevant in the doping-control setting). In such cases, it is our belief that the analyst must confidentially alert the authorities to such a possibility. Authorities are expected to behave ethically towards potentially ill subjects and advise them to seek proper medical advice. In this regard, carefully drafted confidential letters, strong enough to indicate the possibility of an alternative pathological explanation instead of a doping offence, must be supplied by the testing authority. Apart from the two cases here presented, some additional cases where the elevated hCG was linked to tumour have appeared in our laboratory. It is my belief that if all anti-doping laboratories would put similar data together, an important number of subjects have received an early diagnosis thanks to sports drug testing. In fact, anti-doping analysis may sometimes be the first opportunity to reveal pathological signs indicating a need for clinical follow up. A specific guideline on how to deal with hCG findings in sports drug testing would be an important advantage for anti-doping laboratories and sport authorities. Anti-doping scientists should also give an alert when markers other than hCG, compatible both with doping offences and health-related issues, appear.

The activities of anti-doping laboratories can lead to administrative sanctions, resulting in an athlete being ineligible for competition for a period. However, anti-doping results may have implications beyond administrative sanctions. The finding of drug abuse by anti-doping laboratories in countries having strict penalties for drug abusers may give rise to a difficult situation.[15] In such cases, should the anti-doping laboratory immediately inform the authorities, potentially leading to penal sanctions against the subject concerned? Interestingly, the experience presented in case report 2, relating to the blood-transfusion network, shows the opposite kind of problem – when an accredited anti-doping laboratory, in special circumstances, is not allowed to release adverse finding reports to the sport authorities for potential administrative sanctions. The need for harmonization of regulations between public and sport authorities is clear. How the UNESCO Convention (binding on public authorities) and the World Anti-doping Code (binding on sport authorities) could be better integrated is beyond the scope of this paper, but it would obviously be beneficial.

Another lesson from the blood network case is that the analytical aims of sport and national authorities might not be the same and the laboratory must be prepared for a rapid reaction when needed. The example of the rapid development of a suitable methodology to test for rhEPO in blood illustrates this. It is also striking that, at national level, so-called 'experts' without experience in the anti-doping arena may influence judges to take decisions that diverge from what is usual in drug testing and in health and safety. Legal medical experts[22] and anti-doping scientists could be better prepared for such a role. The decision not to consider as a public health infraction the unjustified administration of rhEPO or the inadequate labelling of blood bags is an example of how misleading 'expert' advice may influence decisions. With the probable incorporation of gene doping in the near future, more overlapping between sport sciences and law will occur.[23]

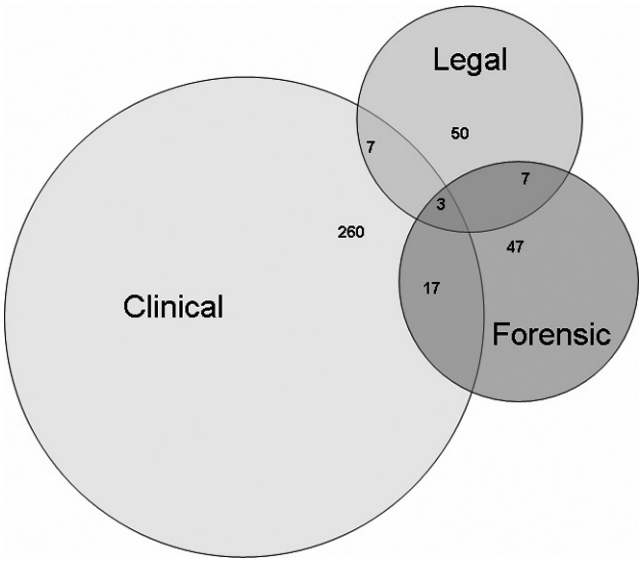

Figure 4. Number of Medline-referred papers dealing with doping and sport with the additional key words 'clinical', 'legal' and/or 'forensic'. These represent 16% of all papers referred to doping and sport.

The deliberate intoxication of a delegation (case report 3) is a dramatic example of the way in which manipulations may sometimes occur as a result not only of the actions of athletes themselves but also as a result of actions by others in the sports world. In the case reported, hair analysis allowed the situation to be clarified, preventing sanctioning of innocent athletes. In this regard, the sentence in the WADA International Standards for Laboratories[1] stating that 'Any testing results obtained from hair, nails, oral fluid or other biological material shall not be used to counter Adverse Analytical Findings or Atypical Findings from urine' must be interpreted in a way that does not preclude finding the best evidence in such situations. In fact, the Society of Hair Testing itself clarifies in its consensus position that 'hair analysis can essentially contribute to doping analysis in special cases, and a positive hair result demonstrates drug exposure during the period prior to sample collection.'[24]

The solution in case report 3 required the collaboration of several laboratories, some of them inside the WADA system and one not belonging to this network. The outcome of that case illustrates that when scientists are faced with a problem and they are eager to collaborate, each performing within his or her own area of expertise, big advances may be made. Successful interlaboratory collaboration is not new in the anti-doping world, with several examples illustrating highly professional behaviour of the scientists involved, especially when testing for big sport events.[25,26]

One outcome of the cases presented is that anti-doping analysts should be open to new issues, methodologies and problems appearing in other related fields.[27,28] Some guidelines from the World Antidoping Agency and Medical Commissions of Sport Organizations would be welcome in order to assist anti-doping laboratories to behave appropriately in borderline situations.

The important relationship between anti-doping specialists and other professionals must be bi-directional. Anti-doping specialists often publish their findings and developments in wider journals, as is illustrated in Figure 4. Clinical, legal and forensic fields must recognize the added value that some results and developments coming from anti-doping laboratories may have, not only because

of the strict quality system operating in anti-doping analysis, which warrants full confidence in the results obtained, but also because information is appearing – and will continue to appear – which could be useful in those other fields.

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