Assessment of the procedures used by the Department of Agriculture and Rural Development (DARD) Northern Ireland in the collection, initial preparation, care, control and analysis of samples taken from steers slaughtered under the OFES scheme and examined for nor testosterone hormone residues.

Report prepared by

Professor Patrick Wall.

5th September 2006

This assessment was prepared at the request of Mr Pat Toal, Permanent Secretary Department of Agriculture and Rural Development.

This assessment was prepared at the request of Mr Pat Toal, Permanent Secretary DARD and is based on my direct observations in three abattoirs, reviews of records, consultations with enforcement staff, laboratory staff, international experts and a literature review, and a laboratory audit undertaken by Dr Peter Kootstra from the EU Community Reference Laboratory as a component of this assessment. This laboratory audit should be read in conjunction with my assessment. (Annex 1)

1 Terms of Reference of Review

I was requested to make observations on DARD's arrangements for the collection and processing of material from bovine animals with special attention to those samples collected from animals slaughtered on farms under the "on farm emergency slaughter" (OFES) scheme and from animals injured during transport from farm to abattoir.

My approach to the assignment was to review the process and procedures from the point of collection of samples for residue analysis until the emergence of the results.

2 Background

In 1988 the EC introduced a prohibition on the use of hormonal substances for animal growth production and has subsequently introduced legislation prescribing the measures to monitor for residues and the actions to be taken on the finding of positive results. (96/22/EC, 96/23/EC, 2003/74/EC. 2005/34/EC). The use of anabolic agents is prohibited for a variety of reasons including possible adverse human health effects, consumer resistance, negative effects on animal welfare and the impact of residues in the environment.

Nor testosterone was first synthesised in the 1950s and initially it was believed to have no natural source. However, it was subsequently shown to occur naturally in boars, stallions, pregnant cows and veal calves. Its presence in adult male bovines is currently deemed illegal under EU law.

On two occasions since January 2004 the hormone, nor testosterone, was identified in bottles, or syringes, on two separate farms in Northern Ireland.

On 10 March 2006 a urine sample was collected from an "On Farm Emergency Slaughter" (OFES) steer presented to ABP Newry, and it screened positive for 17 Alpha - 19-nor testosterone at a low level (1.05 ppb).

In the light of these findings all Official Veterinary Surgeons (OVSs) were instructed to take samples for hormone testing from OFES animals. Between the 10th March and 29th August 2006, 56 out of 117 (about 50%) male OFES animals tested were found positive for nor testosterone.

In addition, 8 out of 32 male animals identified by the OVSs as "casualties" in the lairage also tested positive for nor testosterone.

Two further positive male animals were identified in 55 animals targeted for testing on the basis of the suspicion of the OVSs. One of these was a TB reactor and the other was emaciated.

In contrast, only one positive male animal was found in 279 healthy animals presented for scheduled slaughter who were randomly sampled in 2006 from January 1st to August 29th

Over 600 live animals have been tested as part of investigations on farms of positive OFES cases but no animals positive for nor testosterone have been identified.

One male animal that tested negative for nor testosterone, when sampled on the 21st July as part of an on farm follow up of a positive OFES case, subsequently turned up as an on farm emergency slaughter case on the 10th August and tested positive for nor testosterone.

The level of nor testosterone identified in the positive cases varied from 0.36 to 17.2 parts per billion (ppb).

2.1 Action to be taken on identification of a positive result

A concentration of nor testosterone above the cc alpha, the decision limit of the laboratory assay, in a male bovine is deemed a non compliant result under EU law and the competent authority is duty bound to carry out an investigation on the farm of origin (Article 16 96/23/EC).

The legislation makes the presentation of a positive animal a strict liability matter meaning the onus is on the owner of the animal to provide an explanation for the positive results. However the numbers of animals testing positive and the large number of individual farms involved has created difficulties for DARD if the legislation is to be strictly enforced.

Having regard to the previous findings of nor testosterone on farms in Northern Ireland it was reasonable for DARD to infer that the initial positive results represented illegal use of nor testosterone

Investigations and enforcement actions taken by DARD on the basis of the initial positive results in OFES were in compliance with EU law and based on the state of scientific knowledge at that time.

3 The following question arises;

Is there a physiological explanation for the contrasting results between the OFES and 'normal' slaughter male animals, or is the presence of the hormone at such low levels evidence of illegal administration, deliberate or accidental interference at some stage of the process, or laboratory error?

4.0 I propose to investigate this question by a consideration of the following propositions.

Is there any evidence that:

4.1 On implicated farms

- An illegal product is being used
- Some other therapy is being used that is triggering a positive test result
- Unlicensed anthelmintics, or other products, mixed with hormones are being used on these farms
- Some component of the diet fed to the animals interfered with the test.
- A particular type of injury is associated with the animal being positive for nor testosterone.
- There is an association between the length of time from injury to slaughter and a positive result for nor testosterone
- There is an association between the length of time between on farm slaughter and sampling at the abattoir and the nor testosterone result

4.2 DARD procedures and practices in the abattoir

- Samples could be tampered with from the time of collection on the abattoir floor to dispatch to the laboratory.

4.3 Transport from the abattoirs to the laboratory

- Samples could be interfered with on route to the laboratory either in the vans or in the transport depot

4.4 In the analytical laboratory

- Practices in the laboratory are substandard and interference could occur or false positive results could arise

4.1.1 On the implicated farms.

The visits by DARD officials to the source farms of positive animals did not reveal any substances containing hormones, alternative therapy, unlicensed anthelmintics or dietary

components that might explain the findings. Sampling of over 600 animals on these farms has not revealed any animals positive for nor testosterone.

However there was a delay between the time of OFES, the identification of a positive result and the on farm investigation. This time could allow illegal substances to clear from the systems of cohort animals that it may have been administered to and also the destruction, or removal, of any evidence of illegal substances from the farms. Initially the delay was three weeks but has since been reduced to 10 days. The initial four on farm investigations were unannounced surprise visits, by DARD enforcement staff and police officers, yet nothing untoward was identified.

The EU Hygiene Regulations permit the emergency slaughter of animals on farm if they fulfil the condition:

"An otherwise healthy animal must have suffered an accident that prevents its transport to the slaughter house for welfare reasons."

The herd owner or agent must provide a declaration stating any treatments administered to the animal (Attach a declaration form)

An analysis by DARD staff of the first 49 OFES animals tested (both negative and positive) did not identify any clinical features or injuries specific to those that tested positive.

	Nor testosterone result		
Signs	Positive	Negative	Total
Ataxia/down		1	1
Back injury	4	7	11
Dislocation		1	1
Down/hurt		1	1
back	7	5	12
Fracture		1	1
Hip Injury	6	9	15
Lameness	1		1
Nerve	2	4	6
Paralysis			
Unknown			
Total	20	29	49

However it was not always possible to assess the extent of the injury and its duration prior to slaughter from the paper work provided.

Some of the signs listed would suggest that the animals may not have been emergencies suffering from an accident according to the above definition.

Recommendations

- Farmers need to be made aware of the eligibility criteria for emergency slaughter
- Good husbandry practices should be adhered to in order to reduce the likelihood of on farm accidents to animals and the need for OFES

4.1.2 Certification by Private Veterinary Practitioners (PVP)

The private veterinary practitioner must provide a certificate including the reasons for emergency slaughter, a record of any treatments given, the favourable outcome of ante mortem inspection assessing the animal fit for human consumption and the date and time of slaughter.

Some of the animals had post mortem findings that would suggest that their conditions were not acute.

This finding was conveyed by me to a meeting of representatives of the PVPs in Belfast on the 25th July 2006

"Emergency" implies that slaughter takes place as a result of an event requiring immediate action. An animal suffering from a chronic condition cannot therefore be eligible for slaughter for human consumption.

Recommendation

- The British Cattle Veterinary Association has produced guidance for OFES for veterinary surgeons and this should be followed by PVPs.

4.2 Practices in plant relating to OFES animals

4.2.1 Abattoir operators
Abattoir operators are under no obligation to accept OFES and OFES is not practiced in all EU Member States.

The current trade practice in Northern Ireland is that all abattoirs may accept OFES animals from time to time.

Recommendation

- In the light of the current difficulties in interpreting residue results in OFES animals and the likely unacceptability to consumers and commercial purchasers of Northern Ireland beef of the practice of on farm slaughter, in the interest of consumer confidence and brand protection, consideration should be given by the abattoir operators to reviewing the practice of accepting OFES animals.

4.2.2 The Official Veterinary Surgeon (OVS) in the plant

The Private Veterinary Practitioners should contact the OVS if there is any question about the eligibility of the animal for emergency slaughter.

In addition to ensuring detailed post mortem examination of OFES animals the OVS identifies additional animals in the lairage that may have suffered from accidents in transit and they are deemed "casualties" and sampled.

Recommendation

- The OVS should strictly adhere to the guidelines of the BCVA as to what animals are acceptable for OFES. Animals not fulfilling the criteria should not be accepted and if presented at the abattoir should be deemed unfit for human consumption.

4.2.3 Sampling procedures

Samples of urine are taken on line from the bladder directly into the container by the Meat Inspector (MI) or by a plant operator under the direction of a MI. They can be taken from the bladder within the carcase on the line or after evisceration on the inspection table. The bladder is not always full of urine and the sample volume can vary from several hundred mls to none at all if the bladder is empty. The samples are taken straight to the MIs office by the MI for logging onto APHIS (Animal and Public Health Information System). The MI prepares documents that relate to the sample of urine and these stay with the sample

4.2.4 Security of samples from collection to dispatch from abattoir

Samples are stored in fridges in designated containers, or plastic bags, in a lockable room. Prior to collection they are placed into a transport box with the relevant paper work and the box is sealed. The samples are collected, stored and prepared for dispatch according to DARD's written protocol and this was adhered to in all cases observed and checked.

Even in the most secure systems there is always a possibility that samples could be interfered with. However with the DARD's current procedures and practices this would be extremely difficult and there was no evidence identified of such occurrences.

The fact that positive OFES animals were identified in all red meat abattoirs in Northern Ireland would further suggest it is unlikely that an unscrupulous individual was interfering with the process.

Recommendation

- The relevant security issues in the DARD protocol for taking and handling samples in abattoirs should be extracted from the operations manual for separate circulation to the relevant staff. The instructions should be reinforced by the addition of a requirement to use tamper proof evidence bags and stressing the requirement for two tightly pulled seals on each transport box.

4.3 Transport from abattoir to laboratory

The transport company employs a series of truck drivers each routinely assigned to specific routes. The trucks collect letters, packages and other material for DARD and other agencies and bodies in addition to the sealed transport boxes from the abattoirs. All items collected are taken to a central depot adjacent to Belfast airport for sorting.

There was no evidence observed that boxes were interfered with, or that it would be easy to do so, in transit or in the transport depot.

When the problem with OFES animals positive for nor testosterone began to emerge sample splitting was introduced to check for interference, or substitution, on route. Samples were split in the abattoir and one (Sample 1) travelling to the laboratory via the normal route and another (Sample 2) was collected and brought directly by a laboratory based veterinary surgeon to the laboratory.

4.4 Processing of samples in the Agrifood and Bioscience Institute Laboratory

On receipt in the laboratory campus the samples are taken in by security from where they are delivered to the laboratory reception point. Here they are opened in a secure room and the paperwork is checked and the sample details entered into the laboratory computer.

An examination of sealed boxes received in the laboratory did not reveal any evidence of tampering.

To check that samples were not being interfered with in the laboratory an additional step was introduced with samples being split in the laboratory by the VSD Quality Assurance Unit, one (1A) was processed in the normal way and the other (1B) held securely and tested only if the 1A sample screened positive.

There were no discrepancies between the results obtained with the split samples (1A, 1B and 2)

The samples are subjected to a screening test first and the positives go forward for confirmation.

4.4.1 Analytical capability of the Agrifood and Bioscience Institute Laboratory

The Veterinary Services Division Chemical Surveillance Branch in the Agrifood and Bioscience Institute has been the UK National Reference Laboratory for the analysis of steroid hormones in food producing animals since 1989. It has a track record of excellent work and it is highly regarded internationally.

As I am not an analytical chemist and not competent to access the methods used by the laboratory I requested that an audit of procedures and practices be undertaken by the EU Community Reference Laboratory in Bilthoven, the Netherlands. This was undertaken between August 14-16. The findings concluded that the laboratory is operating to the highest standards and the methods used for screening and confirmation of alpha and beta nor testosterone are validated according to Commission Decision 2002/657/EC. For full report of audit see Annex 1.

4.5 Findings in other Member States

Three positive and three negative results were sent from the Agrifood and Bioscience Institute laboratory to the EU Community Reference Laboratory in Bilthoven. They detected nor testosterone in all three but confirmed it in only one according to protocols outlined in Commission Decision 2002/657/EC.

The same three samples were sent to the French National Reference Laboratory in Nantes where the results were identical to those found in the Agrifood and Bioscience Institute laboratory.

A further 5 positive samples were sent to the EU Community Reference Laboratory at the request of five farmers and all 5 samples were confirmed positive.

The Republic of Ireland's (ROI) National Reference Laboratory in Backweston tested 18 samples taken from male casualties from the ROI and urine from these animals was sent to the Agrifood and Bioscience Institute laboratory. Nor testosterone was identified in 8 and was confirmed in 7 by the ROI laboratory. The Agrifood and Bioscience Institute laboratory confirmed the presence in all of these 8 samples and two of the samples that tested negative in ROI.

Dr Glen Kennedy, Head of the Chemical Surveillance Branch, Veterinary Sciences Division, Agrifood & Biosciences Institute has placed the issue of nor testosterone on the agenda of a meeting of EU Analytical Chemists on 5th October 2006.

Recommendation

- Other member states should be encouraged to test emergency slaughter animals, or casualty animals, for nor testosterone as when positives are found in other jurisdictions this further confirms the findings in Northern Ireland and makes the issue a pan EU one.

5.0 Is there a physiological or natural explanation for the positive samples?

5.1 Dehorning and castration experiments

A number of male cattle (5) were castrated and dehorned at the Agrifood and Bioscience Institute and urine taken from these animals was tested at periods post procedure for nor testosterone. The hypothesis was that the stress of these two procedures, albeit under local anaesthetic, would mimic the stress of a candidate for on farm slaughter. However no elevation of nor testosterone was observed.

5.2 Bacterial contamination of samples during the time between the on farm slaughter and the collection of samples at the abattoir.

It is clear that there is a considerable time lag between the on farm slaughter and the transport of the carcass to the abattoir. During all this time the carcass cannot be regarded as on a par with abattoir slaughtered animals and it is possible that bacteria could be introduced into the urinary bladder and start to metabolise the urine constituents and thus give rise to (chemical) positive results with an innocent explanation.

Seven steers were brought to the Agrifood and Bioscience Institute. They were shown to have nor testosterone free urine. They were killed and urine was collected directly from their bladders at 0, 3, 6 and 24 hours after death (in practice, carcases are supposed to arrive at the abattoir within two hours of slaughter and allowing for the time to process the carcass on line urine samples should be taken within 3 hours of slaughter). The samples were split and either frozen immediately or after holding at 4^0C for 72 hours (the average time taken to get samples from the abattoirs to VSD). No nor testosterone was detected in any samples from these animals suggesting that nor testosterone is not formed after death.

5.3 Meeting with Professor Hubert de Brabander, Dean of the Veterinary Faculty University of Ghent on 23rd August 2006

Professor De Brabander is a world renowned expert on hormone analysis and on the difficulties in distinguishing between exogenous and endogenous anabolic hormones. The scientific consensus has been that there is no evidence that nor testosterone occurs naturally in male cattle. However in the light of the emerging findings from Northern Ireland Professor de Brabander considers that this view will now have to be reconsidered. He cited that as analytic techniques improve and new data emerges,

scientific opinion often changes as there was a time when it was thought that there was no natural source of nor testosterone but subsequently it was discovered to occur naturally in boars, stallions, pregnant ruminants (cattle, sheep & deer) and humans. In most species nor testosterone is endogenous in males and in pregnant females. Until now cattle have been an exception. He cited that in racing stallions the level of nor testosterone increases after exertion and similar observations were made with soccer players after matches. Other examples he cited where scientific opinion changed with new information were the cases of the anabolic agents Boldenone and Zeronal which were at one time believed not to occur naturally. The former was subsequently found to occur in pigs and cattle and the latter in animals consuming grain contaminated with the Fusarium fungus.

He considers that while the issue of nor testosterone in OFES animals is of immediate concern in Northern Ireland it is a Pan-EU issue and should be addressed at this level. The hypothesis that there is some physiological response in animals eligible for emergency slaughter needs to be addressed.

5.4 Meeting with Dr Walter Gillis, Head of hormone Residue control in Belgium

The findings in Northern Ireland were discussed with Dr Gillis. A similar situation does not exist in Belgium as casualty animals are not routinely tested for nor testosterone. Dr Gillis acknowledged the difficulty in differentiating endogenous and exogenous hormones and the consequent problem with strictly applying the legislation throughout the EU.

He agreed to have samples taken from casualty animal for testing for nor testosterone.

6.0 Conclusion

I am satisfied that all reasonable practices and procedures are in place to ensure compliance with the legislation relating to hormone residues by DARD personnel and laboratory staff and that the present methods should be continued.

The finding of nor testosterone in OFES animals has demonstrated the analytical capability of the Agrifood and Bioscience Institute laboratory but has highlighted an inability to distinguish conclusively between exogenous and endogenous nor testosterone. This presents a major problem for DARD in the area of enforcement in OFES but also in female animals where the possibility of pregnancy could be used as an explanation for the presence of nor testosterone.

There is an urgent need to review the parameters of the legislation, which is qualitative in nature, and either set a Threshold 'action level' or develop methods of distinguishing between endogenous and exogenous nor testosterone.

This is clearly a matter that involves the entire European Union.

7.0 Recommendations:-

I. Consider cancellation of OFES

- In the light of the current difficulties in interpreting residue results in OFES animals and the likely unacceptability to consumers and commercial purchasers of Northern Ireland beef of the practice of on farm slaughter, in the interest of consumer confidence and brand protection, consideration should be given by the abattoir operators to reviewing the practice of accepting OFES animals.

II. Ensure the eligibility criteria for OFES are strictly adhered to which should reduce the numbers of animals presented to abattoirs significantly.

- Farmers need to be made aware of the eligibility criteria for emergency slaughter
- Good husbandry practices should be adhered to in order to reduce the likelihood of on farm accidents to animals and the need for OFES
- The British Cattle Veterinary Association has produced guidance for OFES for veterinary surgeons and this should be followed by PVPs.
- The OVS should strictly adhere to the guidelines of the BCVA as to what animals are acceptable for OFES. Animals not fulfilling the criteria should not be accepted and if presented at the abattoir should be deemed unfit for human consumption

III. The relevant security issues in the DARD protocol for taking and handling samples in abattoirs should be extracted from the operations manual for separate circulation to the relevant staff. The instructions should be reinforced by the addition of a requirement to use tamper proof evidence bags and stressing the requirement for two tightly pulled seals on each transport box.

IV. Non compliant results cannot be ignored and article 16 of Council Directive 96/23/EC stipulates the actions to be taken. Initial on farm investigations can take place without enforcement action

V. Consideration should be given to setting a threshold level for enforcement action.

VI. Where illegal treatment has been established enforcement action should be taken according to the Directive (96/23/EC)

VII. Other member states should be encouraged to test emergency slaughter animals, or casualty animals, for nor testosterone as when positives are found in other jurisdictions this further confirms the findings in Northern Ireland and makes the issue a pan EU one.

VIII. To highlight this issue as a Pan EU problem a seminar for the leading analytical chemists and enforcement officers and policy makers should be hosted in NI.

IX. Tests to distinguish between exogenous and endogenous use are in development and there is an urgency for one or more to be field tested in NI and progressed to the stage when the can be used to support enforcement. The two principle ones are the Hair test and the Isotope Ratio test.

Hair test.
Anabolic agents are usually administered as esters which are metabolised to release free hormone which is excreted in the urine. This test detects the steroid ester which is deposited in the growing hair and therefore could distinguish between endogenous production of hormone and illegally administered hormone.

Isotope ratio test
This test is used in detecting performance enhancing hormones in competitive human athletes and in race horses. It looks at the ratio between carbon^{12} and carbon^{13} which is different between exogenous and endogenous hormones.

Acknowledgements

I acknowledge the assistance of the staff of DARD and the Agrifood and Bioscience Institute who responded positively to every request for records, information and access to facilities sought by me during the course of this review.

I acknowledge the assistance of the international experts consulted who shared their knowledge and opinions and tested samples.

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Annex 1

Report and recommendations on an audit by the Community Reference Laboratory RIVM-ARO of Veterinary Sciences Division, Belfast, August 14-16, 2006

Summary

On August 14-16, 2006, P.R. Kootstra, Community Reference Laboratory, RIVM-ARO (NL) visited the laboratory of the Veterinary Sciences Division at Belfast. This laboratory is the UK National Reference Laboratory for anabolic steroids (amongst other compounds).

The purpose of the visit was to perform an audit of the procedure for the screening and confirmation of α and β nortestosterone in urine. The methods were audited against the international standard ISO 17025. The laboratory has a described and operational quality system that complies with the international standard and GLP-principles. The methods for screening and confirmation of α and β nortestosterone are validated according to Commission Decision 2002/657/EC. The quality control of the daily routine analysis is more than adequate. Scientific staff are competent and are skilled to perform the tests. Selected samples were tested in two other laboratories that confirmed the original findings of VSD.

These findings fully confirm in an entirely satisfactory manner the ability of this National Reference Laboratory to perform the analysis of α and β nortestosterone in bovine urine.

Introduction

In March 2006, a urine sample collected from a steer was reported non-compliant for 17α-19-nortestosterone (1.05 ppb). Since then nortestosterone has been confirmed in more than 60 samples. During this period the laboratory developed a new method for the screening of α and β nortestosterone in urine samples. This method is based on a biosensor principle. This specific screening method for of α and β nortestosterone in urine, is performed before the current multi-residue screening method, which is based on LC-MS-MS. The confirmation is performed on a GC-MS-MS system. None of these methods has been accredited to ISO 17025, however the LC-MS/MS and GC-MS/MS procedures have been submitted for accreditation.

The numbers in the text refer to the chapters of the ISO 17025:2005 standard.

The following analytical procedures were witnessed.

-SOP CSD 119V.3 "Detection of α nortestosterone residues in bovine urine by immunobiosensor using immunoaffinity columns"
-SOP CSD 331 V1, " Standard operating procedure for the confirmation of α and β nortestosterone in urine by GC-MS/MS"

The following persons were spoken to:
G. Kennedy, W. Smyth, D. Shortt, A. Hewitt, M. McClean and several other members of the scientific staff.

The expert would like to thank the laboratory and its personnel for their open discussions and their hospitality.

Management requirements
4.1 Organisation

4.2 Quality system

The laboratory has been accredited by UKAS with registration number 2632.
The laboratory is also recognised as GLP-compliant.
The laboratory has a working quality system based on ISO 17025 and GLP standard. The quality manual has some minor flaws. For instance the laboratory states (7.5.2) that calculations and data transfers are subject to appropriate checks in a systematic manner while calculations and data-transfers are checked (e.g. paragraph 5.4.7. below) there is no written procedure describing how this should be done.
There is no cross-reference table available of the chapters to ISO 17025.

4.3 Document control
All SOPs are authorised and each copy is numbered. Documents are periodically reviewed and revised to ensure continuing suitability. No obsolete or uncontrolled documents were found. Staff are using authorised SOPs.

4.13.2 Technical records
Records of original observations and derived data like calculations were readily available to establish an audit trail. The audit trail is documented comprehensively as a result of GLP requirements.

5 Technical Requirements
5.2 Personnel
Training records and qualification of personnel is available and up to date. Every member of scientific staff has a personal record file.

5.3 Accommodation and environmental conditions
The laboratory facilities are sufficient in order to perform the tests. A special room is designed to prevent cross contamination during the preparation of analytical standards.

5.4 Test methods and method validation
5.4.4 Non-standard methods

The methods used by the laboratory are not covered by standard methods and are specified by Commission Decision 2002/657/EC.

5.4.5 Validation of methods
The methods have been validated using SOP CSB 003 for the validation of the screening method and SOP CSB 004 for the validation of the confirmation method. These SOPs are for in-house validation and validation reports were available.
SOP CSD 119V.3, a qualitative screening method has been validated according to CD 2002/657/EC.
SOP CSD 331 V1, is a confirmation method (qualitative) and also suitable for quantification.

Validation reports contain the information in order to reconstruct the validation experiments. Raw data (chromatograms etc.) are kept separated.

5.4.6 Estimation of uncertainty of measurement
For the uncertainty of measurement, the laboratory refers to CCα. If the reported analyte content of a sample exceeds the determined value of CCα, it can be judged non-compliant with a statistical certainty exceeding 99%.

5.4.7 Control of data
Calculations and data transfers are checked. Especially the transfer from results on paper into the database (LIMS) system is not written down but in practice is carried out well.

Back-up systems were not subjected to this audit.

5.5 Equipment
The laboratory has the equipment needed to perform these tests. Calibration programmes are established for balances, pipettes and other relevant equipment.
Analytical instruments are well maintained and records include damage, malfunction, use, maintenance, repair, service, etc.
Instruments are operated by authorised and skilled personnel (see also training).

5.6 Measurement traceability
All equipment used for tests, which may have significant effect on the accuracy of the result, are calibrated. The calibrations are checked on a regular basis and the results are recorded.

5.6.3 Reference standards and reference materials
Standards and reference materials are available and kept in secured storage (GLP compliance).
New standard mixtures are compared with previous standards. However there are no criteria for acceptance or rejection. Every year the results and trends are evaluated

Recommendation
Criteria for acceptance or rejection of freshly made analytical standards should be established.

5.8 Handling of test and calibration items
The procedures for receipt, acceptance, handling, storage and in the case of the analysis for nortestosterone, subsampling, storage and renumbering, are available. Abnormalities from normal and specified conditions are recorded and the customer is consulted for further instructions.
All samples are identified by a unique laboratory number. Due to restrictions of the database, confidential information about the sample is only available to management.

5.9 Assuring the quality of test and calibration results.
5.9.1
At the moment the laboratory is using several methods to monitor the validity of the analytical method. Negative controls are included in each batch to eliminate possible sample contamination. Recovery samples are included to monitor extraction efficiency. At least two unknown recovery samples, spiked by another member of the scientific staff, are analysed in each sequence. Also subsamples are introduced and compared with earlier obtained results. The quality control is more than adequate.
If necessary corrective action is undertaken.

5.9.2b Participation in proficiency testing programmes or interlaboratory comparison
Since 2000 the laboratory participates in proficiency testing programmes organised by Progetto Trieste and RIVM. These results are satisfactory. No Z-scores above 1.7 were observed in the last 5 years (9 samples analysed for nortestosterone). Also no false-positive or false-negative results were found.

Six selected samples were independent tested by the NRL in Nantes (F) and the CRL Bilthoven (NL). The results of these laboratories confirmed the findings of VSD.

Conclusion

The methods are suitable for accreditation.

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SCHEMATIC OVERVIEW OF OPERATIONS ISO/IEC 17025

Name of laboratory:	VSD	Department:	CCU
Name of technical expert:	P.R. Kootstra	Date:	Aug 16, 2006
		Project code:	n/a
Name / Names discussion partner(s):	G. Kennedy, W. Smyth, D. Shortt, A. Hewitt, M. McClean		

Numbers of (groups of) scopes		SOP 119	SOP 331					
Performance of test observed (Y/N)		Y	Y					
4	Management requirements	◘	◘	◘	◘	◘	◘	◘
4.3	Document control	+	+					
4.6	Purchasing services and supplies							
4.9	Control of nonconforming testing							
4.12.2	Technical records	+	+					
5	Technical requirements	◘	◘	◘	◘	◘	◘	◘
5.2	Personnel	◘	◘	◘	◘	◘	◘	◘
5.2.1	Qualification of personnel	+	+					
5.2.2	Education and training	+	+					
5.2.5	Specific tasks							
5.3	Accommodation and environmental conditions	◘	◘	◘	◘	◘	◘	◘
5.3.1	Specifications concerning environmental conditions	+	+					
5.3.2	Control and monitoring of environmental conditions	+	+					
5.3.3	Shielding and separation of neighbouring areas	+	+					
5.3.4	Access	+	+					
5.3.5	Housekeeping	+	+					
5.4	Test methods and method validation	◘	◘	◘	◘	◘	◘	◘
5.4.1	General: working instructions, procedures, standards etc.	+	+					
5.4.2	Selection of test methods	+	+					
5.4.3	Laboratory-developed methods	+	+					
5.4.4	Non-standard methods	+	+					
5.4.5	Validation of methods	+	+					
5.4.6	Estimation of uncertainty of measurement	+	+					

SCHEMATIC OVERVIEW OF OPERATIONS ISO/IEC 17025

Name of laboratory:	VSD	Department:	CCU
Name of technical expert:	P.R. Kootstra	Date:	Aug 16, 2006
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Name / Names discussion partner(s):	G. Kennedy, W. Smyth, D. Shortt, A. Hewitt, M. McClean		

Numbers of (groups of) scopes		SOP 119	SOP 331					
5.4.7	Control of data	◘	◘	◘	◘	◘	◘	◘
5.4.7.1	Control of calculation and data transfer	+	+					
5.4.7.2	Computers and automates equipment							
5.5	Equipment	◘	◘	◘	◘	◘	◘	◘
5.5.1	Availability	+	+					
5.5.2	Calibration program and checks	+	+					
5.5.3	Authorised use / availability of manuals	+	+					
5.5.4	Identification of equipment/software	+	+					
5.5.5	Maintenance of records	+	+					
5.5.6	Handling, transport, storage and maintenance	+	+					
5.5.7	Not proper functioning equipment	+	+					
5.5.8	Calibration status	+	+					
5.5.9	Control of equipment gone outside direct control laboratory							
5.5.10	Intermediate checks	+	+					
5.5.11	Implementation of calibration results/ correction factors	+	+					
5.5.12	Safeguard from adjustments	+	+					
5.6	Measurement traceability	◘	◘	◘	◘	◘	◘	◘
5.6.1	General	+	+					
5.6.2.1	Requirements and considerations for calibration laboratories							
5.6.2.2	Requirements and considerations for testing laboratories	+	+					
5.6.3	Reference standards and reference materials	◘	◘	◘	◘	◘	◘	◘
5.6.3.1 /5.6.3.2	Calibration program and checks	+	+					
5.6.3.3	Intermediate checks							

SCHEMATIC OVERVIEW OF OPERATIONS ISO/IEC 17025

Name of laboratory:	VSD	Department:	CCU
Name of technical expert:	P.R. Kootstra	Date:	Aug 16, 2006
		Project code:	n/a
Name / Names discussion partner(s):	G. Kennedy, W. Smyth, D. Shortt, A. Hewitt, M. McClean		

Numbers of (groups of) scopes		SOP 119	SOP 331					
5.6.3.4	Maintenance, handling, transport and storage	+	+					
5.7	Sampling	◘	◘	◘	◘	◘	◘	◘
5.7.1	Representativity							
5.7.2	Deviations from procedures on client's request							
5.7.3	Registration of relevant data and activities							
5.8	Handling of test items	◘	◘	◘	◘	◘	◘	◘
5.8.1	Transportation, receipt, handling, protection, storage, retention and disposal	+	+					
5.8.2	Identification	+	+					
5.8.3	Check on receipt	+	+					
5.8.4	Avoiding deterioration, loss or damage	+	+					
5.9	Quality control	+	+					
b	Participation in proficiency testing programmes or interlaboratory comparison	+	+					
5.10	Reporting the results	◘	◘	◘	◘	◘	◘	◘
5.10.1	General: punctuality, transparency and unambiguously							
5.10.2	Content of test reports							
5.10.3/ 5.10.4	Additional requirements test reports and Calibration certificates							
5.10.6	Testing and calibration results obtained from subcontractors							
5.10.7	Electronic transmission							
5.10.8	Format of reports and certificates							
5.10.9	Amendments							

Explanation of symbols +

SCHEMATIC OVERVIEW OF OPERATIONS ISO/IEC 17025

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Numbers of (groups of) scopes	SOP 119	SOP 331					

No non-conformities observed	A
Non-conformity category A, as defined in RvA-R2 (RAC)	B
Non-conformity category B, as defined in RvA-R2 (RAC)	NA
Not applicable	blank
Not assessed	→
Non-conformity classification changed to	la
Reference to report lead assessor	