Failed Peak Identification in GC/C/IRMS

The basis of the AAF in Floyd's case is the GC/C/IRMS analysis result.
This AAF is (mainly) based on the isotopic delta-delta values of the testosterone metabolite 5 α-Diol, exceeding the WADA threshold of 3 ‰ as well as LNDD's threshold of 3,8 ‰ , including LNDD's measurement uncertainty of 0,8 ‰.

In order to uphold this conclusion the metabolites of interest in this case must be identified in compliance with the ISL. The WADA Code and the ISL are clear on that:

Code **Article 3.2 Methods of Establishing Facts and Presumptions**
3.2.1 *WADA*-accredited **Laboratories are presumed to have conducted *Sample* analysis and custodial procedures in accordance with the *International Standard* for laboratory analysis.** The *Athlete* may rebut this presumption by establishing that a departure from the *International Standard* occurred. If the *Athlete* rebuts the preceding presumption by showing that a departure from the *International Standard* occurred, then the *Anti-Doping Organization* shall have the burden to establish that such departure did not cause the *Adverse Analytical Finding*.

6.4 Standards for *Sample* Analysis and Reporting. Laboratories shall analyze *Doping Control Samples* and report results **in conformity with the *International Standard* for Laboratories analysis.**

ISL, page 34

5.4.4.3.1 Uncertainty in identification
The appropriate analytical characteristics must be documented for a particular assay.[1] *The* ***Laboratory must establish criteria for identification of a compound at least as strict as those stated in any relevant Technical Document.***

The relevant Technical Document in this case is TD2003IDCR.
It states:

TD2003IDCR

The appropriate analytical characteristics must be documented for a particular assay. The Laboratory ***must establish criteria for identification of a compound****. Examples of acceptable criteria are:*

Chromatographic separation

[1] (As an aside: There is the question if "analytical characteristics" includes identification of a substance. Then LNDD failed because they have no documentation about peak identification in GC/C/IRMS. LNDD's technicians testified there is no SOP for peak identification for essay EC31)

> *For capillary gas chromatography, the retention time (RT) of the analyte shall not differ by more than one (1) percent or ±0.2 minutes (whichever is smaller) from that* ***of the same*** *substance in* ***a spiked urine sample****,* ***Reference Collection sample****, or* ***Reference Material analyzed contemporaneously****. In those cases where shifts in retention can be explained, for example by sample overload, the retention time criteria may be relaxed.*

What the TD2003IDCR states is that one has to compare the retention times of the substances (testosterone metabolites) in the athletes sample with the retention times of the same substance in

1. a spiked urine sample,
2. Reference Material or
3. A Reference Collection sample.

Why did LNDD fail to comply with this paragraph?

1. LNDD did not use a spiked urine sample
2. The Reference Material (Mix Cal AC) did not contain the substances of interest (5 α-Diol, 5ß-Pdiol and Andro). Therefore a comparison of Retention Times between the athletes sample and the Reference Material is not possible.
3. LNDD did not use a Reference Collection.

USADA tried during the CAS Hearing to show that the Blanc Urine is sufficient to serve for peak identification.
The use of Blanc Urine in this very case is not possible because of several reasons.

1. Blanc Urine pool 4 is not a Reference Collection by definition of the ISL.

The ISL (page 36) defines Reference Collection:

> ***5.4.6.2 Reference Collections***
> *A collection of samples or isolates may be obtained from a biological matrix* ***following an authentic and verifiable administration of a Prohibited Substance or Method, providing***
> ***that the analytical data are sufficient to justify the identity of the relevant chromatographic peak*** *or isolate as a Prohibited Substance or Metabolite of a Prohibited Substance or Marker of a Prohibited Substance or Method.*

Those criteria aren't met with Blanc Urine pool 4:

A. "authentic and verifiable administration"

- We don't have a proof of origin of Blanc Urine pool 4

- Blanc Urine pool 4 is a negative control, no administration of the metabolites/substances of interest were conducted to the subjects who gave the urine.
- The subjects who gave the urine weren't examined for medical conditions or the use of medications which could exclude them from providing urine.
- The Blanc Urine pool 4 is a pooled urine of different lab workers and not that of one person

B. "analytical data are sufficient to justify the identity of the relevant chromatographic peak"

- LNDD/USADA couldn't proof that the metabolites/substances in Blanc Urine pool 4 are the substances LNDD claimed them to be. LNDD did not verify by mass spectrometry that the metabolites contained in the blanc urine are in deed the metabolites. What we have is only LNDD's assumption that 5 α-Diol in the Blanc Urine is in deed 5 α-Diol.
- The studies Mongongou claimed she had performed on the Blanc Urine pool 4 weren't provided. Because of the importance of this studies/verification of the Blanc urine pool 4 one would expect LNDD would have provided them if they really exist.
- The documents provided- even in the declarations for the CAS Hearing by Buisson- are the pages LNDD0309 and LNDD0310 (SOP E-P-32).

LNDD's Blank Urine Characteristic (LNDD0309/LNDD0310)

What is LNDD309/LNDD0310?
It is a characteristic of the negative control urine.

Which information is provided on this SOP E-P-32 (LNDD0309/0310)?

Under point 1 we learn about the codification and the collection time of the Blanc Urine.

- It was collected between 6.12.2005 and 12.12.2005 and the code is BluP 4.

Under point 2 we learn about pH and density of this BluP4.

- It has a specific gravity of 1,023 and a pH of 5,58.

Under point 3 we learn that BluP4 underwent a GC/MS screening analysis on the MSD18 instrument.

- The purpose of this screening analysis by GC/MS was to establish estimates of concentrations for the metabolites.

- It was a screening analysis and not a confirmation analysis.

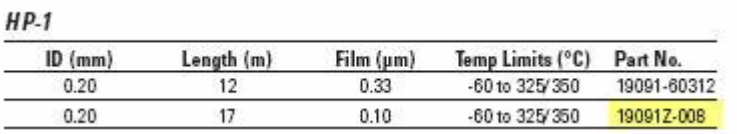
- The sample preparation and the conditions of analyses in screening and confirmation procedures are different and not comparable.

ISL, page 19:
5.2.4.3 Urine confirmation testing
All Confirmation Procedures must be documented and meet applicable uncertainty requirements. The objective of a Confirmation Procedure is to ensure the identification and/or quantification and to exclude any technical deficiency in the Screening Procedure. Since the objective of the confirmation assay is to accumulate additional information regarding an adverse finding, ***a Confirmation Procedure should have greater selectivity/discrimination than a Screening Procedure***.

- This screening was performed on the MSD18 instrument which has a HP1 column.

- The column used in the MSD18 GC/MS instrument is an Agilent 19091Z-008 column.(USADA0045)

- The polarity of this column is non polar and therefore different of the DB17 column used in the Isoprime 1 instrument.



HP-1

ID (mm)	Length (m)	Film (µm)	Temp Limits (°C)	Part No.
0.20	12	0.33	-60 to 325/350	19091-60312
0.20	17	0.10	-60 to 325/350	19091Z-008

Fig.1: Agilent GC Column Selection Guide, p.81

- There is no indication or proof that the metabolites of the Blanc Urine Pool 4 were identified by mass spectrometry.

So the pupose of this analysis was not the identification of the metabolites but the quantification of the supposed metabolites.
Even if LNDD performed mass spectrometry (what we don't know, because we weren't provided with the proof this was done) to identify the very metabolites they did it on a GC/MS instrument with a different column. The use of the different columns in the MSD18 GC/MS instrument and the Isoprime 1 instrument would make a comparison of the retention times or pattern between GC/MS and GC/C/IRMS impossible because the elution order may have changed.

Under point 4 (LNDD0310) we learn about the isotopic values of the supposed six metabolites.

- The BluP 4 was analysed 3 times on the Isoprime 1 instrument and the mean delta values of those metabolites were calculated as well as the delta-delta values of the testosterone metabolites subtracted by the endogenous reference compound.

- Those data were obtained under the "optimal" conditions of the Isoprime 1 instrument.

We do not even know if the conditions of the Isoprime 1 instrument in the analysis of the BluP 4 were the same as used in the analysis of sample 995474.
There is no reference to a SOP on LNDD0309/0310, i.e.to M-AN-41, where the instrumental setup of the Isoprime 1 for IRMS analysis of BluP 4 is described.

If the argument would be made that the metabolites in the BluP 4 were identified only in the GC/C/IRMS analysis, then following TD2003IDCR there must have been a Reference Solution, like Mix Cal Acetate, containing ALL six metabolites otherwise it is the same issue as with sample 995474.
And this is: there is no identification of 5 α-Diol, 5ß-Pdiol and Andro in GC/C/IRMS analysis.

Furthermore it is impossible that LNDD had used Reference Standard solutions like Mix Acetate or Mix Cal Acetate for compound identification in Blu P4 because the Reference standard solutions Mix Acetate (used for GC/MS) and Mix Cal Acetate (used for GC/C/IRMS) were first time approved in January resp. Mai 2006. (see LNDD0442). There were no standard solutions containing the reference metabolites in place before this date.

LNDD	MODE OPÉRATOIRE	Codification : M-EXMIX-05 Version : B Date :09/05/2006 2 / 2

METHODE DE PREPARATION DU MIX ACETATE ET DU MIX CAL ACETATE

Action	Personne concernée	Date	Signature
rédigé par	Cynthia MONGONGU	09/05/2006	
vérifié par	Corinne BUISSON	09/05/2006	
vérifié par	Aurélie LAURENT	09/05/2006	
approuvé par	Jacques DE CEAURRIZ	09/05/2006	

EVOLUTIONS

N° Version	Motif	Date
A	Création du document.	17/01/2006
B	ajout du mix cal acétate	09/05/2006

Fig.2 LNDD0442

This argument is supported by the documents which are showing the reference standards used for Mix Cal Acetate.
The reference standard documents have a stamp on in it providing the date when those reference standards have been prepared:

Code	Substance	Date	Reference
Cal Acetate 1	5 α-AC (Internal Standard)	17.5.2006	LNDD0296
Cal Acetate 2	5 ß Pdiol AC	17.5.2006	LNDD0299
Cal Acetate 3	Etio	17.5.2006	LNDD0302
Cal Acetate 4	11-Ketoetio AC	21.4.2006	LNDD0305

This table shows that the reference standards contained in the Mix Cal Ac were prepared approximately 4-5 month after analysis of Blu P 4.

Summary of LNDD0309/0310 for BluP 4:

1. The columns used in GC/MS and GC/C/IRMS analysis of BluP 4 were different. Therefore a comparison of retention times or pattern between those two instruments is obsolete.
2. There is no proof of identification of the target metabolites in GC/MS analysis for BluP 4, neither by retention time (compared to Reference Standards of that of the same substance or else) nor by mass spectrometry.
3. There is no proof of identification of the target metabolites in GC/C/IRMS for BluP 4 by comparison of the BluP 4 to reference standards of that of the same substance.

Bottom-line:

LNDD did not provide any proof that the metabolites in the BluP4 were identified according to the TD2003IDCR.
Therefore BluP 4 cannot serve as a Reference Collection.

2. Even USADA did not claim BluP 4 is a Reference Collection

Furthermore, even USADA and USADA's witnesses never claimed that BluP 4 is a Reference Collection (In the Hearing Transcripts the term "Reference Collection" is not mentioned once by USADA or witnesses for USADA!).

3. The use of a Reference Collection is inappropriate in existence/presence of Reference Standards by ISL

Leave out the above; the ISL states that "Reference Standards should be used for identification, if available."
We know through the Discovery Documents that LNDD is in possession of reference standards for the metabolites 5 α-Diol (LNDD0287), 5ß-Pdiol (LNDD0278) and Andro (LNDD0284).
The ISL paragraph 5.4.4.2.1. continues: "If there is no reference standard available, the use of data or sample from a validated Reference Collection is acceptable."
By claiming that BluP 4 is a Reference Collection and used for compound identification LNDD also violated this ISL paragraph because the use of a validated Reference Collection (which BluP 4 is not) is only acceptable (emphasis added) if there is no Reference Standard available.

ISL, page 32

5.4.4.2 Validation of Methods
5.4.4.2.1 Confirmation methods for Non-threshold Substances must be validated. Examples of factors relevant to determining if the method is fit for the purpose are:

…

□□*Standards. Reference standards should be used for identification, if available.* ***If there is no reference standard available****, the use of data or sample from a validated Reference Collection is acceptable.*

Conclusion

For all those reasons LNDD did not identify the metabolites 5 α-Diol, 5ß-Pdiol and Andro, which are the metabolites on which the AAF in this case is based. Without identification nobody can be sure what was measured by LNDD, there is no proof that the peaks in the GC/C/IRMS chromatograms contain the alleged metabolites, therefore there is no AAF.