EOTTD meeting Cork May 13-14, 2016

Report of the Laboratory working Group discussion session

The predominant issue discussed was the reproducibility and comparability of the hCG measurements obtained by the different assays used by the EOTTD members. It was suggested to organize an inquiry amongst the members in order to get a complete overview on the different hCG assays and the platforms used, and on how members organize internal and external quality assessment. EQA samples could be prepared and sent to a number of institutes to demonstrate the urgent need to harmonize assays or assay results within the EOTTD. It was discussed whether this will yield us new information, but we all agreed that it certainly will benefit the awareness of members of the issue. Although members of the working group believe that LC- tandem mass spectrometry will likely become the ultimate approach in the future to quantify a number of different molecular forms of hCG in blood simultaneously, they expect this new methodology will take quite some years before it will be implemented, pan-Europe, in a routine laboratory setting. Therefore, most of the meeting time of the working group was devoted to discussions on the features and the requirements of the presently available and new antibody based (sandwich) assays. Ideally, an assay would detect all molecular forms of hCG in an equimolar ratio (i.e 100% crossreactivity for all forms), in order to assure the presence of certain isoforms will not be under- or overestimated. Presently used assays do not meet this criterion. The Nijmegen radioimmunoassay for instance overestimates the free beta hCG component by a factor of 10 (1000% cross-reactivity), while the assay from Charing Cross' hospital has a slightly lower recognition for this component of around 76-78% (Harvey *et al.,* 2010, Sturgeon *et al.,* 2009). Each of the many commercially available assays has its own "recognition pattern" for the molecular hCG forms, resulting is strongly different hCG results even when measuring the same set of samples.

Following the groups discussions several (theoretically) possible approaches to the organization of hCG measurement in European countries emerged.

1) All hCG assays are performed within one European lab facility for EOTTD, and preferably also for all other, institutes.
2) All members use the same assay and performance is monitored by an independent EQA organization.
3) All members continue to use their own assays and assay results are harmonized by an external organization.
4) All members continue their present way of hCG assessment using internationally established cut-offs or regression corridors.
5) All members will continue their present way of hCG assessment, but establish their own cut-off values or regression corridors.
6) Each EOTTD country will have an hCG assay platform that is used across all the centres for that country. The assays would be EOTTD-approved by our working group.

The working group thinks that option 1 is not a pragmatic option and will not happen. Option 2 would be nice, but in general in most countries hCG is assessed by the clinical chemical laboratories on a random access platform. Lab managers' choice for a type platform is based on many parameters and certainly not predominantly on hCG performance. Option 3 is, theoretically speaking, hard to achieve, but it has recently been shown to be a successful approach for another multifactorial hormone like Growth Hormone (Ross *et al.*, 2014). In the Netherlands all laboratories use harmonized GH values. This needs the development of commutable well-defined hCG samples and quite some lab investigations. Option 4 - the status quo is, in our view probably the least attractive. Option 5 is simply unlikely to be achievable. Option 6 requires further discussions within each of the member states but was, like 3, considered a good approach.

The question then (option 6) is which assay to use? Mologic in Amsterdam last year presented their new antibody based hCG assay and in the present Cork meeting showed more details of the performance of the assay. The working group evaluated (on paper) the assay based on the presented data and is eager to learn more about the assay. A major point of concern is the lack of data on long term stability, robustness and availability of the assay. A particular advantage of the Nijmegen and Charing's Cross RIAs is that they have performed for decades and have shown tremendous stability and Antibody resources permit further use in both centres for quite a number of years. So the working group considers the Mologic assay as potentially very interesting, but before continuing within this, several things have yet to be worked out. EOTTD should, for instance, be guaranteed that the reagents (antibodies, label) should be made available to the group, in order to assure continuity in case Mologic terminates its activities.

Summarizing the Cork working group meeting, the working group proposes to the EOTTD board:

- To consider organizing a short -hCG directed- inquiry amongst EOTTD members in order to get more insight into the present situation regarding the types of hCG assays and EQA schemes used.
- To monitor development in literature regarding hCG assessment by LCMS. Nijmegen in near future intend to start development of such an assay
- To further discuss within the group the development of a harmonization sample.
- To start discussion with Mologic on mutual requirements for a long term collaboration, i.e use of their assay. This discussion should be led by 2 members of working group supported by a member of the EOTTD board. If this negotiation is successful, pilot evaluations should be done in 2-3 EOTTD institutes for a prolonged time in order to study long term assay stability, make comparisons with presently used assays, and to perform some general analytical validation studies, according to NCCLS protocols (i.e. EP5, EP6, EP9).

The working group awaits further recommendations by the board. On behalf of the lab working group.

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