Report

WHO Consultation on International Standards for *in vitro* Clinical Diagnostic Procedures based on Nucleic Acid Amplification Techniques (NAT)

Geneva, Switzerland
22-24 April 2002

WORLD HEALTH ORGANIZATION
Blood Safety and Clinical Technology
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1. OPENING REMARKS

In vitro diagnostic tests have become increasingly important tools of modern medicine. With the proliferation of biological diagnostic test kits, the need for standardization is becoming essential to the regulation and quality control of these tests, and this is now an international global issue.

Dr. Jean Emmanuel, Director of the WHO Blood Safety and Clinical Technology Department opened the meeting and welcomed the participants, with further remarks made by Dr. Elwyn Griffiths, Coordinator of Quality Assurance and Safety of Biologicals. They summarized the status of the current WHO reference materials for NAT which were established by the WHO Expert Committee on Biological Standardization over the past five years. These materials were designed and prepared to assist in the validation of nucleic acid based diagnostic tests applied to the viral safety testing of blood products, such as those for hepatitis C virus, hepatitis B virus, and the AIDS virus.

The objective of this Consultation was to exchange information regarding the role of reliable and consistent quantitative NAT in the clinical aspects of hepatitis and AIDS, and regarding the future needs for standardization of in vitro clinical diagnostic NAT procedures. More specifically, the participants were charged to consider the current WHO international reference materials as they relate to the use of NAT for monitoring changes in viral load of patients undergoing antiviral therapy and, if necessary, to define the characteristics of international reference materials that would be appropriate for this purpose. Dr. Van Aken and Dr. Decker were appointed chairman and rapporteur, respectively, for the meeting.

2. EXPERIENCE WITH CURRENT NAT INTERNATIONAL STANDARDS

Presentations by the participants covered experiences with current NAT International Standards for HIV, HCV and HBV from the perspectives of the WHO International Laboratories for Biological Standards, manufacturers, regulatory authorities, and clinicians. The presentations included observations with NAT for both blood product safety testing and in vitro clinical diagnostic testing.

Drs. Minor and Lelie presented the observations of the two WHO International Laboratories for Biological Standards. Dr. Minor gave a summary of development of International Standards within WHO and stressed the historical importance of the quantitative values assigned to these preparations. Dr. Lelie reminded the group that standardization and
control of NAT for viral safety of blood products focus primarily on method sensitivity. For blood safety applications, quantitative values have often been measured by limiting dilution to estimate the lowest reproducible limits of sensitivity. He described some of the problems that have surfaced during the use of NAT, such as the variability found among the NAT methods when they are used to estimate the number of genome equivalents (geq) in an international unit (IU) of HCV, HIV or HBV.

Drs. Madej and Mimms expanded on this variability of methods, showing that the different nucleic acid based tests may differ more than 2-fold when measuring the number of genome equivalents per IU. The group was also reminded that the IU for the WHO NAT reference materials for HCV, HBV, and HIV differ from each other relative to the number of geq each represents. For instance, one IU of HCV RNA equals approximately 4 geq of HCV, while one IU of HBV DNA is approximately 7 geq of HBV, and one IU of HIV RNA equals approximately 1 geq of HIV. This led to a discussion on whether unitages should be defined in terms of geq rather than IU and what the advantages and disadvantages of these expressions would be in relation to the clinical needs.

Drs. Pawlotsky and Esteban described their clinical experiences with the use of NAT for monitoring therapy, particularly for hepatitis C. Changes in viral load values correlate well with patients’ response or non-response to therapy and provide early and objective data regarding the response. Dr. Pawlotsky reminded the group that one should not try to associate nucleic acid concentration directly with relative viral infectivity since the two values may or may not correlate with one another. He and Dr. Esteban believe physicians are more concerned with consistency of the methods and are less concerned with whether unitage is expressed in IU/ml or geq/ml. Dr. Pawlotsky showed data on series of patient specimens that were tested using two different NAT methods where divergent profiles were sometimes obtained. This suggests that other method-dependent variables can influence the quantitative measurements. Dr. Esteban stressed that knowledge of data supporting a sustained response to therapy improves patients’ morale and increases their adherence to a difficult regimen, whereas early knowledge of a non-response allows physicians to offer alternative treatments sooner.

Dr. Nübling reviewed recent European experience with NAT applications for safety testing of blood products. He covered the regulatory approach of the Paul Ehrlich Institute (PEI), and this also reflects the approach taken by accredited notified bodies in Europe. He outlined the essential requirements for diagnostic tests as defined by the European IVD Directive and as covered under Common Test Specifications (CTS). They include test reliability, performance, risk analysis and traceability. He pointed out the role of NAT international standards during the development of NAT for viral safety of blood products in Europe. There remains a need for continued harmonization of NAT standards within the scientific community, and this need will grow because of the increasing use of quantitative NAT in patient care. Dr. Nübling described some of the characteristics of synthetic nucleic acids and how they could eventually serve as international standards. While such reference materials could have many advantages, he noted that they would not necessarily serve to validate the complete NAT systems. For instance, they would bypass the extraction procedures.

Drs. Hewlett and Yu outlined the regulatory structure of the FDA for reviewing and approving donor screening tests and in vitro diagnostic tests in USA. They described their experiences with the FDA approach to licensing of NAT kits for HIV and hepatitis. Since
there are now multiple technologies that fall under the umbrella of “NAT”, there is an increased need for harmonizing standards and for standardized reporting of data to facilitate comparison of results. CBER/FDA has developed well-characterized panels that are used for validation purposes with NAT for HCV and HIV. Dr. Hewlett and Dr. Yu expressed positive views toward considering synthetic nucleic acids as reference materials and as possible future international standards.

3. DEVELOPMENT AND REPLACEMENT OF NAT INTERNATIONAL REFERENCE PREPARATIONS

Dr. Hendricks reviewed issues to consider in the development of International Standards for NAT vis-à-vis applications for monitoring of viral load. Foremost, such reference materials should perform consistently. Ideally, they should contain full lengths of the nucleic acid sequences found most often in the viruses in vivo, they should perform in hybridization assays like the native materials, and they should be readily available. Other items to consider for these standards include: concentrations for therapeutic monitoring (10^6-10^7 gEq would be a suitable range), the nature of the target (biochemical integrity, whether full length or fragments), reproducibility (vial-to-vial, lot-to-lot), traceability, continuity of unitage, real-time stability, absence of interfering substances, and absence of other adverse influences of matrix components. In terms of integrity of the materials, he and others expressed concerns about possible adverse effects of lyophilization and reconstitution on performance of the standards. Dr. Hendricks described some of the advantages in using synthetic nucleic acids for future WHO standards. Among the advantages is the possibility of using independent methods to measure the standards quantitatively (e.g., phosphate). This information could have a bearing on estimates of the genome equivalents in such preparations. It may be anticipated that future reference standards for some of the non-blood borne viruses will depend on synthetic nucleic acids, and efforts that are put forth on synthetics here could provide a blueprint for standards to follow.

Concerns were expressed that synthetic nucleic acids do not have viral protein capsids and would not represent the true target. For example, the extraction procedure of an assay might be compromised. However, it was argued that the primary purpose of a reference standard is to provide accurate unitage and not necessarily to serve also as a control for the extraction procedure. Some synthetic nucleic acids for HCV are currently being used by manufacturers in-house, and some of these could be candidates for WHO studies. Dr. Hendricks described his experience with synthetic HCV RNA of approximately 2.4 kb. The group agreed that full-length synthetic nucleic acids would be preferable to partial transcripts. Dr. Yu suggested that a full length transcript of HCV RNA may be available from NIH/CBER.

Drs. Saldanha and Heath described the development of current NAT reference materials for HCV, HBV and HIV that were prepared under the auspices of WHO. They reviewed the specific steps involved in the actual manufacture of the International Standards and the WHO Collaborative Studies. Many of these activities will be required when replacement of NAT reference materials is undertaken either because current supplies become exhausted or approaches to new standards are determined. Dr. Heath presented examples of the statistical approaches that were used during the WHO Collaborative Studies on the NAT International Standards. Dr. Saldanha outlined a protocol for the next WHO Collaborative Study to be initiated for confirming the replacement lot of the current HCV Standard when it is exhausted. Dr. Saldanha also proposed that this or a second WHO Collaborative Study be
done to investigate the performance of candidate synthetic nucleic acids as compared with the current biological WHO reference materials.

The group reviewed the desired and acceptable characteristics of WHO International Reference preparations that should be used in the standardization of nucleic based tests as applied both for quantification of viral load and for viral safety testing procedures. Since the group felt that synthetic nucleic acids offer some advantages as materials that could meet the desired characteristics of future International Standards, this subject was discussed at length. As a result of this discussion, an approach was agreed upon.

4. CONCLUSIONS AND WAYS FORWARD

- Synthetic target nucleic acids are to be considered as material for International Standards. There is a need for data about the performance of such substances as compared with the presently used biological NAT International Standards for HIV, HCV and HBV.

- ILC was invited to come forward with HCV synthetic nucleic acid materials that would be used in a feasibility study of these materials as candidate international reference preparations. They were invited to plan and carry out the feasibility study.

- If the results of the feasibility study with synthetic nucleic acids so indicate, one or more candidate synthetic nucleic acids will be included in a WHO Collaborative study for HCV RNA. This WHO collaborative study is targeted for the end of the year.

- Because the current batch of the WHO NAT International Standard for HCV will soon be depleted, a WHO collaborative study to confirm the stability of a second batch (batch BB) of the same material will be performed. Dr. Saldanha presented the outline for this study.

- Experience in the development of the HCV synthetic reference material will be used as a model for HBV and HIV. Dr. Hewlett proposed that work on the development of HBV and HIV synthetic materials also be initiated. She proposed to the group that CBER could initiate some work with synthetic HIV RNA.

- Future studies must also consider possible genotype variations for all three viruses.

As to details regarding the study of synthetic nucleic acid materials, representatives of the manufacturers should first obtain their respective corporate approvals regarding issues of Intellectual Property (IP) and the provision of the selected synthetic materials. ILC will identify potential candidate materials, define their characteristics and design a feasibility study to assure that the candidates are appropriate for use with the different nucleic acid based methods. This information will be provided to a Working Party composed of two representatives from the ILC (names to be determined), two representatives from the WHO International Laboratories for Biological Standards (Drs. Lelie and Saldanha), two representatives from regulatory authorities (Drs. Nübling and Hewlett), and Dr. Ana Padilla, WHO. Dr. Padilla will coordinate the Working Party.

The report of this Consultation will be presented by Dr Padilla to the next meeting of the WHO International Working Group on the Standardization of Genomic Amplification...
Technologies for the Virological Safety Testing of Blood and Blood Products (SoGAT) which will take place in Athens on the 30 May 2002.

CAALENDAR:

End May 2002: Selection of materials, design and conduct of the feasibility study by ILC.

September 2002: Review of results of feasibility studies; plan approaches to the protocol of a WHO Collaborative study for HCV RNA, by the NAT Working Party.

May 2003: Interest was expressed by the Consultation Group that all information of the work performed in this exercise be completed in 2003 and reported to WHO SoGAT.
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