Distribution: General
English only

Report

Working Group on International Reference Materials for Diagnosis and Study of Transmissible Spongiform Encephalopathies (TSEs):

Fourth Meeting

Geneva, Switzerland
29-30 April 2002

WORLD HEALTH ORGANIZATION
Blood Safety and Clinical Technology
April 2002

CONTENTS

WHO Working Group on International Reference Materials for Diagnosis and Study of Transmissible Spongiform Encephalopathies (TSEs) Fourth Meeting

WHO Headquarters, Geneva
29-30 April 2002

Report

1. INTRODUCTION

The Fourth Meeting of the WHO Working Group on International Reference Materials for Diagnosis and Study of Transmissible Spongiform Encephalopathies (WHO TSE Working Group) was held at WHO in Geneva on 29-30 April 2002. The meeting was opened by Dr Jean Emmanuel, Director of the Blood Safety and Clinical Technology Department with further remarks made by Dr Elwyn Griffiths, Coordinator of Quality Assurance and Safety of Biologicals, WHO. The importance of suitable biological reference materials to improve the understanding and control of human TSEs was stressed. They reminded the TSE Working Group that biological reference materials are needed to assure that *in vitro* diagnostic tests and tests to detect infectious agents are robust enough and that—in spite of the unusual difficulty involved in working with TSE—tests for TSEs should be subjected to the same degree of critical scrutiny as are other tests. The high cost in effort and funds needed to develop such tests was recognized and they expressed their gratitude to all participants for the work of the Group. It was stated that these efforts would help to harmonize laboratory diagnostic tests and manufacturing process validation methods for TSEs, reminding the Group that WHO biological reference materials require international consensus.

2. WHO COLLABORATIVE STUDY WITH HUMAN BRAIN-DERIVED MATERIALS

2.1 In vitro studies

Eight participants produced data confirming the presence of abnormal scrapie-type prion protein (PrP^{Sc}) in each of the three Creutzfeldt-Jakob disease (CJD) candidate biological reference materials described in the previous report. Six participants examined the preparations by western blot (Prusiner, Bolton et al. 1982), one by conformation-dependent immunoassay (Safar, Wille et al. 1998; Safar, Cohen et al. 2000) (CDI), and one by a DELFIA-based system (MacGregor, Hope et al. 1999; Barnard, Helmick et al. 2000). (Five initial participants did not return final results in time to incorporate them into the report as presented) The data confirmed that the level of PrP^{Sc} in the preparation from one sporadic case of CJD is lower than that in the other case of sporadic CJD; the preparation from the vCJD case contains the highest level of PrP^{Sc}. When the results were expressed as the minimal mass of brain tissue required to detect PrP^{Sc}, all western immunoblots and the original CDI method had roughly similar sensitivities. A "sandwich" CDI recently modified by Dr. Vey and colleagues, using an antibody that captures PrP, was substantially more sensitive than the other methods for detecting PrP^{Sc}. Drs. Vey and Safar explained the potential advantages of the new sandwich CDI. The Working Group agreed to work with WHO staff to announce in an appropriate scientific journal that these materials are now available as WHO reference materials for use as calibrants by qualified investigators.

2.2. Use of human brain-derived materials

The vCJD preparation contained PrP^{Sc} with the electrophoretic glycotype pattern typical of this form, while the two sporadic CJD preparations both had a mixture of the two glycoforms described in the previous meeting report. The Working Group concluded that such mixed glycotypes are probably more common than was previously recognized but that such preparations are not ideal for future studies requiring well-characterized strain types. These preparations should nonetheless be satisfactory for use as calibrants in studies requiring quantification of PrP^{Sc}. The existing vCJD preparation is considered to be suitable for all *in vitro* and *in vivo* studies.

Prof. James Ironside agreed to coordinate a sub-group that will attempt to identify brains or parts of brain suitable to prepare reference materials containing single pure PrP^{Sc} glycoforms.

2.3. Transmission studies

Each biological reference material should be assayed in titration for infectivity in susceptible animals. This information is essential for complete characterization of the proposed reference materials. Dr. Manson intends to initiate transmission studies of these reference materials in transgenic mice (Manson 1999). She requested assistance of the WHO in obtaining funding to support these experiments. Other laboratories may also participate, depending upon the availability of funding. Assay in a variety of animals, including PrP-bovinized (Scott, Safar et al. 1997) and PrP-humanized (Telling, Scott et al. 1995) transgenic mice and BSE-susceptible conventional mice (especially RIII mice (Bruce, Will et al. 1997)) and in primates was suggested.

3. WHO STUDY ON TERMINOLOGY FOR TYPES AND SUBTYPES OF HUMAN PrP^{Sc}

Of the 12 participants, 11 have submitted full or partial reports of the nine samples provided by Prof. Collinge (including two of type 1, two of type 2, three of type 3, and two of type 4, according to Prof. Collinge's glycotyping classification (Collinge, Sidle et al. 1996)) and four samples by Prof. Gambetti (including two of type 1 and two of type 2, according to the glycotyping classification of Dr. Parchi and Prof. Gambetti (Parchi, Castellani et al. 1996)).

Prof. Budka concluded that glycotyping results were relatively uniform for PrP^{Sc} prepared by both Prof. Collinge's and Prof. Gambetti's western blotting methodologies. Most laboratories, however, had difficulty applying Prof. Collinge's classification scheme, although in the hands of Prof. Collinge and Dr. Wadsworth the results were reproducible. Laboratories had no difficulty in using the classification scheme of Dr. Parchi and Prof. Gambetti consistently. The successful use of Prof. Collinge's system requires careful optimization of sample preparation, including

control of the content of Cu^{++} and Zn^{++} and the use of uniform electrophoretic gels with exceptionally high resolving capacity (Wadsworth, Hill et al. 1999).

The Working Group concluded that the Parchi-Gambetti type 1 PrP^{Sc} includes Collinge types 1 and 2 PrP^{Sc}, that Parchi-Gambetti type 2A is equivalent to Collinge type 3, and that Parchi-Gambetti type 2B is equivalent to Collinge type 4 (vCJD).

4. BLOOD REFERENCE MATERIALS

Drs. Rohwer, Baron, MacGregor and Barrowcliffe presented reviews of possible approaches to reference materials for blood diagnostic tests. There was general agreement to adopt a "staged" or sequential approach to offering reference materials appropriate for use as calibrants by investigators developing diagnostic or screening tests for human blood and for studies of TSE agent clearance by various manufacturing processes: (1) infected animal brain material and human brain material suitable for calibrating spikes for TSE detection protocols (low-titered material) and for agent clearance studies (high-titered material); (2) infected animal blood (rodent blood—probably from hamsters with 263K scrapie (Brown, Rohwer et al. 1998; Brown, Cervenakova et al. 2001) and sheep blood if further characterization proves that model to be reliable (Houston, Foster et al. 2000)); (3) human blood from subjects with clinical CJD. Of the three kinds of potentially useful reference materials suitable for studies with blood, only the first is feasible to be offered now. The second would be extremely expensive using hamster blood, and uncertainties remain concerning sheep blood. For the third kind of material—human blood—there is still no convincing proof of concept that either abnormal PrP or infectivity is present in the blood of persons with CJD (Brown 2001).

The Working Group agreed that, in spite of some concern about relevance for human blood, a reference material comprised of a well studied strain of some animal TSE agent should be made available for validation of assays where the agent was to be used in spiking experiments; it was recommended that a crude brain suspension from animals infected with the 263K strain of hamster-adapted scrapie would be highly suitable for that purpose. In addition, human citrated plasma spiked with the existing vCJD reference brain suspension and another plasma spiked with the preparation of sporadic CJD brain containing the higher level of PrP^{Sc} will be prepared and made available.

Normal and vCJD human spleen extracts were suggested as being potentially more relevant reference materials than are brain-derived materials for spiking experiments of human blood; spleen suspensions will be prepared in sucrose by a modification of the same method used for the human brain-derived reference materials. Human blood itself was not recommended as a suitable biological reference material.

A sub-group on human blood reference materials comprised of Drs. Barrowcliffe, Giulivi and Rohwer and van Engelenburg will prepare a detailed plan of action to develop agreed-upon candidate materials suitable for use in facilitating the development of blood-based diagnostic tests. The plan of action—to include identification of source materials, required characterization of materials, and projected funding needs—will be sent to the Secretariat within a month.

The Working Group also concluded that blood from TSE-infected rodents is of great value because it contains endogenous infectivity and is probably relevant to potential human blood-borne infectivity. Because of the logistic difficulties in preparing volumes of rodent blood large enough to serve as a reference material and lack of agreement about blood components to be stored and methods of separation, the Working Group did not recommend developing a rodent blood reference material at this time.

Blood from sheep experimentally infected with BSE or scrapie agents may eventually prove suitable to provide large amounts of relevant reference material. Development of sheep blood TSE reference material will be investigated but cannot be recommended yet.

5. ACTIVITIES RELATED TO ANIMAL-DERIVED TSE REFERENCE MATERIALS

Dr. Matthews reported that some bovine material potentially suitable for preparation as a BSE reference is available at the Veterinary Laboratories Agency, UK. However, he emphasized that all requirements for material must be specified in detail before any material can be provided.

Mr. Schimmel warned the participants that the PrP-based rapid in vitro tests currently used in the EU for BSE screening (Deslys, Comoy et al. 2001; Grassi,

Comoy et al. 2001) have performed satisfactorily when used with fresh bovine brain specimens, but frozen brain specimens have unexpectedly yielded both false positive and false negative test results. These appear to reflect some poorly understood effects of differences in conditions of brain storage and homogenization. In addition, he noted that the distribution of PrP^{Sc} in bovine brain stems is very heterogeneous, although it fortunately tends to be laterally symmetrical; this requires great care in selecting areas of brain for sampling. Several other participants reported having had similar experience with human brain tissues, suggesting that human PrP^{Sc}, like bovine PrP^{Sc}, may be less stable on storage than is rodent-adapted scrapie-infected material.

Dr. Safar reviewed the potential advantages offered by transgenic mice expressing or over-expressing various engineered prion proteins (Telling, Scott et al. 1995; Scott, Safar et al. 1997) for establishing abundant supplies of well-documented homogeneous TSE biological reference material and for sensitive, rapid and reproducible assays of infectivity. Such models may become especially important to study strains of TSE agent, since strains appear to maintain unique biological properties on serial passage in transgenic mice. It seems likely that useful biological reference materials will eventually be prepared as suspensions of tissue from transgenic mice infected with various strains of TSE agent. The possible effect of a murine matrix (differences between human and mouse tissue that might influence the performance of a murine reference material as a calibrant for tests of human tissue) must be carefully considered. At the moment, transgenic mice have not been made available to WHO.

6. OTHER BUSINESS

The Working Group plans to meet once a year or twice if progress justifies convening a second meeting. In order to facilitate the progress of the work, two sub-groups were formed. The sub-group on human-brain-derived reference materials will

provide an inventory of available CJD tissues that might be suitable for preparing pure PrP^{Sc} glycotype reference materials and will submit a plan to evaluate and select candidate brain tissues. The sub-group on blood reference materials will prepare a detailed plan for further work to be submitted to the WHO Secretariat for review by those participants of the TSE Working Group involved in blood safety.

REFERENCES

Barnard G, Helmick B, Madden S, Gilbourne C and Patel R. The measurement of prion protein in bovine brain tissue using differential extraction and DELFIA as a diagnostic test for BSE. Luminescence 2000;15(6): 357-62.

Brown P. Creutzfeldt-Jakob disease: blood infectivity and screening tests. Semin Hematol 2001;38(4 Suppl 9): 2-6.

Brown P, Cervenakova L and Diringer H. Blood infectivity and the prospects for a diagnostic screening test in Creutzfeldt-Jakob disease. J Lab Clin Med 2001;

137(1):5-13.

Brown P, Rohwer R G, Dunstan B C, MacAuley C, Gajdusek D C and Drohan W N. The distribution of infectivity in blood components and plasma derivatives in experimental models of transmissible spongiform encephalopathy. Transfusion 1998;38(9): 810-6.

Bruce M E, Will R G, Ironside J W, McConnell I, Drummond D, Suttie A, McCardle L, Chree A, Hope J, Birkett C, Cousens S, Fraser H and Bostock C J. Transmissions to mice indicate that 'new variant' CJD is caused by the BSE agent. Nature 1997;389(6650): 498-501.

Collinge J, Sidle K C, Meads J, Ironside J and Hill A F. Molecular analysis of prion strain variation and the aetiology of 'new variant' CJD. Nature 1996;383(6602): 685-690.

Deslys J P, Comoy E, Hawkins S, Simon S, Schimmel H, Wells G, Grassi J and Moynagh J. Screening slaughtered cattle for BSE. Nature 2001;409(6819): 476-8.

Grassi J, Comoy E, Simon S, Creminon C, Frobert Y, Trapmann S, Schimmel H, Hawkins S A, Moynagh J, Deslys J P and Wells G A. Rapid test for the preclinical postmortem diagnosis of BSE in central nervous system tissue. Vet Rec 2001;149(19): 577-82.

Houston F, Foster J D, Chong A, Hunter N and Bostock C J. Transmission of BSE by blood transfusion in sheep. Lancet 2000;356(9234): 999-1000.

MacGregor I, Hope J, Barnard G, Kirby L, Drummond O, Pepper D, Hornsey V, Barclay R, Bessos H, Turner M and Prowse C. Application of a time-resolved fluoroimmunoassay for the analysis of normal prion protein in human blood and its components. Vox Sang 1999;77(2): 88-96.

Manson J C. Understanding transmission of the prion diseases. Trends Microbiol 1999;7(12): 465-7.

Parchi P, Castellani R, Capellari S, Ghetti B, Young K, Chen S G, Farlow M, Dickson D W, Sima A A, Trojanowski J Q, Petersen R B and Gambetti P. Molecular basis of phenotypic variability in sporadic Creutzfeldt-Jakob disease. Ann Neurol 1996;39(6): 767-78.

Prusiner S B, Bolton D C, Groth D F, Bowman K A, Cochran S P and McKinley M P. Further purification and characterization of scrapie prions. Biochemistry 1982;21(26): 6942-50.

Safar J, Cohen F E and Prusiner S B. Quantitative traits of prion strains are enciphered in the conformation of the prion protein. Arch Virol, Suppl 2000;(16): 227-35.

Safar J, Wille H, Itri V, Groth D, Serban H, Torchia M, Cohen F E and Prusiner S B. Eight prion strains have PrP(Sc) molecules with different conformations. Nature Med 1998;4(10): 1157-65.

Scott M R, Safar J, Telling G, Nguyen O, Groth D, Torchia M, Koehler R, Tremblay P, Walther D, Cohen F E, DeArmond S J and Prusiner S B. Identification of a prion protein epitope modulating transmission of bovine spongiform encephalopathy prions to transgenic mice. Proc Natl Acad Sci U S A 1997;94(26): 14279-84.

Telling G C, Scott M, Mastrianni J, Gabizon R, Torchia M, Cohen F E, DeArmond S J and Prusiner S B. Prion propagation in mice expressing human and chimeric PrP transgenes implicates the interaction of cellular PrP with another protein. Cell 1995;83(1): 79-90.

Wadsworth J D, Hill A F, Joiner S, Jackson G S, Clarke A R and Collinge J. Strain-specific prion-protein conformation determined by metal ions. Nature Cell Biol 1999;1(1): 55-9.

Annex 1

WHO Working Group on International Reference Materials for Diagnosis and Study of TSEs
4th Meeting

WHO Headquarters, Geneva
29 (9h00) - 30 (18h00) April 2002
Room D

Agenda

Day 1

1. Opening Remarks

2. WHO collaborative study: *in vitro* studies on human brain derived materials
   Draft report. Dr P. Minor

3. Transmission studies: Dr. J. Manson; Prof. Collinge

4. Use of human brain derived materials: Dr P. Minor

5. WHO Study on terminology for types and subtypes of Human PrP^{Sc}:
   Draft report. Dr. H. Budka / Dr P. Minor

Day 2

6. Blood Reference Materials:
   Dr. R. Rohwer; Dr. H. Baron; Dr I. Mc Gregor; Dr T.W. Barrowcliffe

7. Activities in relation to animal TSE reference materials:
   Progress towards the provision of animal reference materials: Dr D. Mathew
   Preparation and standardization of materials for test evaluation: Dr H. Schimmel

8. Rodent adapted strains of TSE agents: Dr J Safar

9. Scrapie standards: Dr P Minor

10. Any other business

Annex 2

WHO Working Group on International Reference Materials for Diagnosis and Study of TSEs: 4th Meeting
WHO Headquarters, Geneva,
29-30 April 2002, Room D

List of Participants

Dr D. Asher (Rapporteur)
FDA Center for Biologics Evaluation and Research (CBER)
Office of Blood Research and Review
Division of Emerging and Transfusion-Transmitted Diseases
Laboratory of Special Pathogens
FDA HFM-313
1401 Rockville Pike,
Rockville, Maryland 20852-1448
USA

Dr H. Baron
Senior Director
Prion Research
Aventis Behring S.A.
46 Quai de la Rapée
F-75601 Paris Cedex
France

Dr T. W. Barrowcliffe
Head, Hematology Division
National Institute for Biological Standards and Control
Blanche Lane, South Mimms
Potters Bar
Hertfordshire EN6 3QG
UK

Dr Catherine Bergeron
Associate Professor of Pathology
University of Toronto
Tanz Neuroscience Building
6 Queen's Park Crescent West
Toronto, Ontario M5S3H2
Canada

Dr J. P. Brandel
INSERM Unité 360
Hôpital la Salpêtrière
47 Bd de l'Hôpital
75651 Paris Cedex 13
France

Professor H. Budka
Professor of Neuropathology
Institute Director
Institute of Neurology, University of Vienna,
Austrian Reference Centre for Human Prion Diseases
AKH 4J, POB 48, A-1097 Wien
Austria.

Dr Larisa Cervenakova
The Jerome Holland Laboratory
American Red Cross
15601 Crabbs Branch Way
Rockville
Maryland 20855
USA

Professor J. Collinge
MRC Prion Unit, Department of Neurogenetics
Imperial College School of Medicine At St Mary's, Norfolk Place,
London W2 1PG
UK

Dr S. Collins
Department of Pathology
The University of Melbourne
Parkville, Victoria 3010
Australia

Dr Jillian K. Cooper
Prion Unit
National Institute for Biological Standards and Control
Blanche Lane, South Mimms
Potters Bar
Hertfordshire EN6 3QG
UK

Dr D Dormont
Service de Neurovirologie
Direction des Sciences du Vivant
Département de Recherche médicale
Centre d'Etudes Nucléaires (CEA)
B.P. 6
92265 Fontenay aux Roses Cedex
France

Dr A. Giulivi
Associate Director
Bureau of Infectious Diseases
Blood-borne Pathogens Division
Laboratory Centre for Disease Control
Health Protection Branch
LCDC Building, AL 0601E2
Ottawa, ON K1A 0L2
Canada

Dr M. Glatzel
Institute of Neuropathology
University Hospital of Zurich
Schmelzbergstrasse 12
CH-8091 Zurich
Switzerland

Dr Luisa Gregori
Head, Biochemistry Section
Laboratory of Molecular Neurovirology
Veterans Affairs Medical Center
Medical Research Service 151
10 N Greene St
Baltimore, Maryland 21201
USA

Dr M. H. Groschup (Unable to attend)
Federal Research Centre for Virus Diseases
of Animals
Director BSE Group
Boddenblick 5a
17498 Insel Riems, Mecklenburg-
Vorpommern
Germany

Dr M. W. Head
National Creutzfeldt-Jakob
Disease Surveillance Unit
Western General Hospital, Crewe Road
GB-Edinburgh EH4 2XU

Dr J. Hope
V.I. Technologies, Inc.
134 Coolidge Avenue
Watertown, MA 02472
USA

Dr Nora Hunter (unable to attend)
Institute for Animal Health, AFRC
Ogston Building
West Mains Road
GB-Edinburgh, EH9 3JF

Professor J.W. Ironside
Consultant Neuropathologist
National Creutzfeldt-Jakob
Disease Surveillance Unit
Western General Hospital, Crewe Road
GB-Edinburgh EH4 2XU

Professor T. Kitamoto
Professor of Pathological Neurology
Faculty of Medicine
Tohoku University
2-1 Seiryou-chou
Aoba-ku, Sendai
Japan

Professor H. A. Kretzschmar
Reference Center for Prion Diseases and
Neurodegenerative Diseases(DGNN)
CJD Surveillance Unit München/Göttingen
Institute of Neuropathology
Marchioninistr. 17
81377 München
Germany

Ms Victoria Lewis
Department of Pathology
The University of Melbourne
Parkville, Victoria 3010
Australia

Dr Jean Manson
Institute for Animal Health, AFRC
Ogston Building, West Mains Road
GB-Edinburgh, EH9 3JF

Dr D. Matthews
TSE Programme Manager
Veterinary Laboratories Agency
New Haw, Addlestone
Surrey, KT15 3NB
UK

Dr I. McGregor
Scottish National Blood Transfusion Service
Protein Fractionation Centre
Ellen's Glen Road
Edinburgh EH17 7QT
UK

Dr P. Minor (Chairman)
National Institute for Biological Standards and Control
Blanche Lane, South Mimms
Potters Bar
Hertfordshire EN6 3QG
UK

Dr P. Parchi
Department of Neurological Sciences
University of Bologna
Via Ugo Foscolo 7
40123 Bologna
Italy

Dr. P. Piccardo
Indiana University School of Medicine
Department of Pathology (Neuropathology)
635 Barnhill Drive MS A-142
Indianapolis IN 46202-5120
Indiana
USA

Professor M. Pocchiari (Rapporteur)
Registry of Creutzfeldt-Jakob Disease
Laboratory of Virology
Istituto Superiore di Sanita
Viale Regina Elena 299
I-00161 Rome
Italy

Dr R. G. Rohwer
Division of Molecular Neurovirology
Laboratory
Medical Research Service 151
VA Maryland Health Care System
10 N Greene St
Baltimore
Maryland 21201
USA

Dr J. Safar
Institute of Neurodegenerative Diseases
University of California, San Francisco
HSE 774, Box 0518
San Francisco
CA 94143-0518
USA

Mr H. Schimmel
IRMM/GRC
European Commission
Rettieseweg
2440 Geel
Belgium

Dr S. Sethi
Reference Center for Prion Diseases and Neurodegenerative Diseases(DGNN)
CJD Surveillance Unit München/Göttingen
Institute of Neuropathology
Marchioninistr. 17
81377 München
Germany

Dr S. Chen
Assistant Professor of Pathology
Division of Neuropathology
Case Western Reserve University
2085 Adelbert Road
Cleveland OH 44106
USA

Dr C. Soto
Serono Pharmaceutical Research Institute
14 Chemin des Eaux
1228 Plan Les Ouates
Switzerland

Mr C. Stenland
Bayer Corporation
P.O. Box 13887
85 T.W. Alexander Drive
Research Triangle Park
NC 27709-3887
USA

Dr Thomas Stroebel
Institute of Neurology, University of Vienna,
Austrian Reference Centre for Human Prion Diseases
AKH 4J, POB 48, A-1097
Wien
Austria.

Dr F. Tagliavini
Istituto Nazionale neurologico Carlo Besta
Via Celoria 11
20133 Milano
Italy

Dr Marc Turner
Edinburgh Blood Transfusion Centre
Royal Infirmary of Edinburgh
Lauriston Place
Edinburgh
UK

Dr F.A.C. van Engelenburg
Plesmanlaan 125
1066 CX Amsterdam
P.O. Box 9190
1006 AD Amsterdam
the Netherlands

Dr M. Vey
Prion Research
Aventis Behring S.A.
46 Quai de la Rapée
F-75601 Paris Cedex
France

Dr J.D. Wadsworth
MRC Prion Unit, Department of Neurogenetics
Imperial College School of Medicine At St Mary's, Norfolk Place,
London W2 1PG
UK

SECRETARIAT

Dr J.C. Emmanuel, Director, BCT
Dr E. Griffiths, Coordinator, VAB/QSB
Dr F.-X. Meslin, Coordinator CDS/CSR/APH
Dr K. Morimoto, EDM/QSM
Dr L. Noel, Coordinator BCT/BTS
Dr A. Padilla, BCT/QSD (**Secretary**)
Ms C. Ponce, BCT/QSD
Dr L. Rago, Coordinator EDM/QSM
Dr L. Pergami, CDS/CSR/APH