Annex 6

REQUIREMENTS FOR MENINGOCOCCAL POLYSACCHARIDE VACCINE

(Requirements for Biological Substances No. 23)

Addendum 1980

INTRODUCTION

The WHO Expert Committee on Biological Standardization, in 1975, adopted the Requirements for Meningococcal Polysaccharide Vaccine, which were published in its twenty-seventh report (*1*, p. 50). At that time it was recognized that the technology involved in the quality control of such vaccines was changing rapidly but the Requirements were written because meningococcal meningitis is a serious problem in many countries and national health authorities, particularly in the developing countries, had requested guidelines for quality control.

During the year following the formulation of the Requirements, progress had been made in the preparation of polysaccharides of higher molecular weight (greater potency) for both Group A and Group C. Furthermore, a greater purification was achieved by the removal of endotoxin so that it became possible to inoculate 10 times the quantity of the polysaccharides into rabbits without a significant rise in temperature. Accordingly, an addendum to the Requirements to take these developments into consideration was published in the twenty-eighth report of the Expert Committee on Biological Standardization (*2*, p. 52).

Further amendments were made and published in the twenty-ninth report of the Expert Committee on Biological Standardization (*3*, p. 94) in order to take into consideration the need to add lactose as a stabilizer and thereby prevent thermal depolymerization. It was

also reported that much research was in progress on the production of polysaccharides from Groups Y, 29E, and W135, but infections by these Groups occurred at a lower frequency than those by Group A and Group C.

Such research has now shown that the polysaccharides from each of these three additional Groups can be isolated and stabilized and they have been characterized. The incidence of infections by Groups Y and W135 strains are increasing in some countries. They now account for more than 10% of the cases and are particularly virulent. It is important, therefore, to make provision for their inclusion in the vaccine, and in so doing a number of the requirements will need amendment. It is recommended that where the infectious agent causing meningitis has not been grouped a quadraspecific vaccine should be used.

In order to avoid reference to several publications, the amendments published in the twenty-eighth and twenty-ninth reports of the Expert Committee on Biological Standardization are included in the present amendments.

AMENDMENTS

Amendment 1

Part A, section 1.1 *International name and proper name*

Replace the whole section by the following:

"The international name shall be '*Vaccinum meningitidis cerebrospinalis*' followed in parentheses by the serogroup specificity, thus: polysaccharide Group A, Group C, Group Y, and/or Group W135. The proper name shall be the equivalent of the international name in the language of the country of origin.

The use of the international name should be limited to vaccines that satisfy the requirements formulated below."

Amendment 2

Part A, section 1.2 *Descriptive definition*

Replace the first sentence by the following:

"*Vaccinum meningitidis cerebrospinalis* shall consist of purified Group A, Group C, Group Y, and/or Group W135 meningococcal polysaccharides."

Amendment 3

Part A, section 3.1.1 *Strains of* Neisseria meningitidis

Replace the whole section by the following:

"The strains of *Neisseria meningitidis* Group A, Group C, Group Y, and Group W135 used for preparing polysaccharide shall be approved by the national control authority. They shall also have been shown to be capable of producing polysaccharide known to be safe and effective in man.

The following strains have been shown to be suitable: for Group A polysaccharide—A1, M1027; for Group C polysaccharide C11, C2241; for group Y polysaccharide—Y (Slaterus), 6306Y, IM2261; for Group W135—W135 6308, S. 4383, IM2263."

Amendment 4

Part A, section 3.4

Replace the section heading by the following:

"**Chemical and immunochemical requirements for lots of purified polysaccharide**"

Amendment 5

Part A, section 3.4.1 *Protein content*

Replace the whole section by the following:

"Each lot of purified polysaccharide shall contain less than 10 mg of protein per gram of polysaccharide for Groups A and C organisms and less than 50 mg of protein per gram of polysaccharide for Groups Y and W135 as determined by the method of Lowry et al. [reference: LOWRY, O.H. ET AL. *Journal of biological chemistry*, **193**: 265 (1951)] using bovine plasma albumin as a reference or other methods approved by the national control authority."

Amendment 6

Part A, section 3.4.2 *Nucleic acid content*

Replace the whole section by the following:

"Each lot of purified polysaccharide shall contain less than 10 mg of nucleic acid per gram of polysaccharide for Groups A and C organ-

isms and less than 20 mg of nucleic acid per gram of polysaccharide for Groups Y and W135 as determined by spectrophotometry, assuming that the absorbance of a 10 g/l solution of nucleic acid contained in a cell 1 cm wide at 260 nm is 200."

Amendment 7

Part A, section 3.4.3 *O-acetyl content*

Replace the whole section by the following:

"The *O*-acetyl content of the polysaccharides from the different strains shall be equal to or greater than 2.0 mmol/g of polysaccharide for Group A, 1.5 mmol/g of polysaccharide for Group C, and 0.3 mmol/g of polysaccharide for each of the Groups Y and W135. The *O*-acetyl content shall be determined by the method of Hestrin [reference: HESTRIN, S. *Journal of biological chemistry,* **180**: 249 (1949)], using acetycholine chloride as the reference preparation."

Amendment 8

Part A, section 3.4.5 *Sialic acid content*

Replace the whole section by the following:

"The sialic acid content of the purified polysaccharide, calculated as free *N*-acetylneuraminic acid (molecular weight 309), shall be not less than 800 mg/g of the dry weight of the isolated product for Group C and not less than 560 mg/g for each of the Groups Y and W135. The determination shall be done by the method of Svennerholm [reference: SVENNERHOLM, L. *Biochimica et biophysica acta,* **24**: 604 (1957)] using sialic acid, *N*-acetylneuraminic acid, as the reference preparation."

Amendment 9

Part A, section 3.4.6 *Molecular size*

Replace the paragraph in large type by the following:

"The molecular size of each lot of purified polysaccharide shall be estimated by gel filtration using Sepharose 4B or Sepharose CL-4B. Chromatography shall be carried out in a solvent having an ionic strength of 0.2 mol/kg. The molecular size shall be determined by

measuring the recovery of the polysaccharide eluted before a K_D of 0.50 is reached. At least 65% of the Group A, 75% of the Group C, 80% of the Group Y, and 80% of the Group W135 polysaccharides shall be recovered from the column before a K_D value of 0.50 is reached."

Amendment 10

Part A

Add the following new section:

"3.4.7 *Test for serological identity and specificity*

Each lot of purified monospecific polysaccharide shall be tested for serological identity and specificity by a test approved by the national control authority.

Tests such as haemagglutination inhibition, immunoprecipitation, counter-immunoelectrophoresis, radioimmunoassay, ELISA, or rocket electrophoresis have been shown to be suitable.

The monospecific polysaccharide of Group A or C or Y or W135 organisms shall be shown to contain less than 1% by weight of any heterologous *N. meningitidis* polysaccharides during production by the manufacturer.

An immunoprecipitation test has been shown to give reliable results. In some countries the haemagglutinin inhibition test has been used but it has been shown to give some unreliable results. An immunochemical test is therefore to be preferred. Tests such as radioimmunoassay, ELISA, rocket or counter-current immuno-electrophoresis have been shown to be suitable."

Amendment 11

Part A, section 3.5 *Preparation of final bulk*

Amendment 2 of the 1977 Addendum remains unaltered, as follows:

"The final bulk shall be prepared either from a single lot of purified polysaccharide or from several pooled lots. The polysaccharide shall be dissolved under aseptic conditions in a sterile solution suitable for freeze-drying and free of pyrogenic substances. A stabilizer shall be added, the substance used and its concentration being subject to approval by the national control authority.

The mixture shall be sterilized by membrane filtration.

Membranes with a pore size of 0.22 μm have been found satisfactory.

A suitable stabilizer is lactose at a concentration of 2.5–5.0 mg per human dose of polysaccharide. It is important to calculate the concentration of lactose on the basis of the anhydrous lactose molecule and not on the basis of the pentahydrate, which is the form most commonly available."

Amendment 12

Part A, section 3.5.1 *Sterility test on the final bulk*

Delete the whole section.

Amendment 13

Part A, section 3.5.2 *Test for serological specificity*

Renumber as section 3.5.1 and replace by the following:

"3.5.1 *Test for serological identity*

The final bulk of the monospecific or polyspecific meningococcal polysaccharides shall be tested for serological identity and for the absence of heterologous polysaccharides by the test described in Part A, section 3.4.7."

Amendment 14

Part A, section 4, *Filling and drying*

Replace the last sentence by the following:

"The stabilized dried vaccine shall be stored at a temperature of 2–8 °C."

Amendment 15

Part A, section 5.3 *Concentration of polysaccharide*

Replace the whole section by the following:

"At least one final container shall be checked to determine that it contains the stated amount of polysaccharide. Monospecific vaccines

shall contain at least 75 mg of phosphorus per gram of Group A polysaccharide, 750 mg of sialic acid per gram of Group C polysaccharide, or 520 mg of sialic acid per gram of the polysaccharides from Group W135 or Group Y. In multispecific vaccines in which the polysaccharides cannot be distinguished chemically this shall be done by a quantitative immunochemical test approved by the national control authority. The final container shall contain the declared content of each Group-specific polysaccharide ± 30%, using the purified polysaccharides incorporated in the vaccine as a reference."

Amendment 16

Part A, section 5.5.1 *Pyrogenicity test*

Replace

"Group A vaccine, 0.025 μg
Group C vaccine, 0.025 μg
combined Groups A and C vaccine, 0.050 μg"

by the following:

"Group A vaccine, 0.025 μg
Group C vaccine, 0.025 μg
Group Y vaccine, 0.025 μg
Group W135 vaccine, 0.025 μg
A bispecific vaccine, 0.05 μg
A trispecific vaccine, 0.075 μg
A quadraspecific vaccine, 0.10 μg"

Amendment 17

Part A, section 5.6 *Estimation of molecular size*

Replace the whole section by the following:

"The molecular size of the polysaccharide in at least one final container from each filling lot shall be determined by Sepharose 4B or Sepharose CL-4B gel filtration as outlined in Part A, section 3.4.6. For both monospecific and multispecific vaccines in which the polysaccharides cannot be distinguished chemically, the criteria of Part A, section 3.4.6 shall apply.

When the column eluates are evaluated by an immunochemical method (see Part A, section 5.3) the major peak of each group polysaccharide shall elute with a K_D value of 0.40 or less."

Delete footnote 1.

Amendment 18

Part A, sections 5.7 and 5.8

Replace these sections by the following:

"**5.7 Test for residual moisture**

The moisture content of the dried material shall be determined as indicated in Part A, section 3.4. The method used for the determination of the moisture content shall be approved by the national control authority.

The test shall be performed on 1 vial per 1000 up to a maximum of 10 vials but not less than 5 vials taken at random throughout the filling lot. The average residual moisture shall be not greater than 2.5% and no vial shall have a residual moisture content of 3% or greater.

5.8 Storage

The stabilized freeze-dried vaccines shall be stored at a temperature of 2–8 °C."

Amendment 19

Amendment 8 of the 1977 Addendum remains unaltered as follows:

"Section 8, on labelling, should reflect the fact that for stabilized vaccines it is no longer necessary to store Group A polysaccharide at –20 °C or lower. Delete the last paragraph and replace by the following:

Furthermore, the label on the container, or the label on the carton enclosing several containers, or the leaflet accompanying the container shall contain the following additional information:

— a statement that the stabilized vaccines shall be stored at 2–8 °C;
— a statement that the reconstituted stabilized vaccine shall be stored at 2–8 °C and shall be discarded if not used during the day on which it is reconstituted."

Amendment 20

Replace Amendment 9 of the 1977 Addendum by the following:

"10.1 Storage conditions

The manufacturer shall recommend such conditions of storage and shipping as will ensure that the vaccine conforms to these requirements until the expiry date as stated on the label. The Group A, C, Y, and/or W135 vaccine shall be stored at a temperature between 2 °C and 8 °C."

Amendment 21

Replace Amendment 10 of the 1977 Addendum by the following:

"10.2 Expiry date

The expiry date for dried bulk polysaccharide when stored at –20 °C or below shall not be more than 5 years from the date of harvest. The expiry date of the stabilized vaccine in the final containers when stored at 2–8 °C shall be not more than 2 years from the date of issue. The manufacturers shall provide data to confirm the stability of their vaccine.

A further test for molecular size may be made and if it is found to be satisfactory the national control authority may wish to allow a further storage period of one year."

Amendment 22

Amendment 11 of the 1977 Addendum remains unaltered as follows:

"In the Appendix to the Requirements, section 3, subsection (4), delete the last sentence of paragraph (*d*), together with footnote 1."

Amendment 23

Part B, section 3. *Reactivity and immunogenicity of vaccine in man*

Replace the third paragraph of the requirements printed in small type by the following:

"Samples of sera from each subject taken immediately prior to injection and again 2–4 weeks after immunization should be assayed for bactericidal antibodies. The bactericidal assay should be performed with paired sera from each subject in serial two-fold dilutions against a suitable strain for each of the polysaccharides contained in the vaccine. The antibody titre should be expressed as the reciprocal of the highest dilution that effects 50% or greater killing of the test organisms. The antibody titres of the sera from at least 90% of the subjects should show a four-fold or greater rise after immunization. If the sera from less than 90% but more than 80% of the subjects show such a rise, one re-test of the product may be allowed, but in such a case the sera from at least 90% of all subjects in the two tests combined should show a fourfold or greater antibody increase."

ACKNOWLEDGEMENTS

Acknowledgements are due to the following experts for their comments and advice and for supplying additional data relevant to the formulation of these modifications:

Dr F. André, Smith Kline RIT, Rixensart, Belgium

Dr F. Arminjon, Mérieux Institute, Charbonnières-les-Bains, France

Dr R. Austrian, University of Pennsylvania, Philadelphia, PA, USA

Dr G. Ayme, Mérieux Institute, Charbonnières-les-Bains, France

Dr E. C. Beuvery, National Institute for Public Health, Bilthoven, Netherlands

Dr G. Y. Causse, Chief, Bacterial and Venereal Infections, WHO, Geneva, Switzerland

Dr P. Crooy, Smith Kline RIT, Rixensart, Belgium

Professor S. G. Dzagurov, Tarasevič State Institute for the Standardization and Control of Medical Biological Preparations, Moscow, USSR

Dr C. E. Frasch, Bureau of Biologics, Food and Drug Administration, United States Public Health Service, Bethesda, MD, USA

Dr C. B. Gerichter, Ministry of Health, Jerusalem, Israel

Dr E. Gotschlich, Rockefeller University, New York, USA

Dr C. Guthrie, Production Director, Commonwealth Serum Laboratories, Parkville, Victoria, Australia

Dr J. Henrichsen, State Serum Institute, Copenhagen, Denmark

Dr M. Hilleman, Merck Institute for Therapeutic Research, West Point, PA, USA

Dr M. Huet, National Health Laboratory, Montpellier, France

Professor D. Ikić, Institute of Immunology, Zagreb, Yugoslavia

Professor L. Jännes, Chief, Production Laboratory, Central Public Health Laboratory, Helsinki, Finland

Dr J. Jeljaszewicz, National Institute of Hygiene, Warsaw, Poland

Professor G. Laurell, University of Uppsala, Sweden

Dr P. Lemoine, Chief, Vaccine Control Section, Institute of Hygiene and Epidemiology, Brussels, Belgium

Dr R. de Neys, Smith Kline RIT, Rixensart, Belgium

Professor F. Oberdoerster, State Institute of Serum and Vaccine Testing, Berlin, German Democratic Republic

Professor F. Pocchiari, Director-General, Higher Institute of Health, Rome, Italy

Mr K. Pope, Merck Sharp & Dohme, Zürich, Switzerland

Dr G. del Real, Llorente Institute, Madrid, Spain

Dr J. B. Robbins, Bureau of Biologics, Food and Drug Administration, United States Public Health Service, Bethesda, MD, USA

Dr E. J. Ruitenberg, National Institute for Public Health, Bilthoven, Netherlands

Professor M. Saletti, SCLAVO Institute for Sera and Vaccines, Siena, Italy

Dr R. Schneerson, Bureau of Biologics, Food and Drug Administration, United States Public Health Service, Bethesda, MD, USA

Mr J. R. Thayer, National Biological Standards Laboratory, Canberra, Australia

Dr R. H. Tiesjema, National Institute for Public Health, Bilthoven, Netherlands

Dr A. F. Woodhour, Merck Sharp & Dohme, West Point, PA, USA

REFERENCES

1. WHO Technical Report Series, No. 594, 1976.
2. WHO Technical Report Series, No. 610, 1977.
3. WHO Technical Report Series, No. 626, 1978.