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Annex 3
Recommendations for the production and control of group C meningococcal conjugate vaccines
(Addendum 2003)

At its fifty-second meeting the Expert Committee on Biological Standardization adopted the Recommendations for the production and control of group C meningococcal conjugate vaccines, which were published in its report (*1*). The Committee agreed with a proposal to draft an addendum on serological assays to evaluate the immune responses to these vaccines and to review the current recommendations in the light of data emerging from the United Kingdom following the introduction of the vaccine, especially data related to the demonstration of immunological memory.

The addendum is intended to serve as an Appendix to the already adopted Recommendations.

Appendix 1

Evaluation of the immunogenicity of group C meningococcal conjugate vaccines

Each manufacturer should evaluate different lots of single component or combined meningococcal C conjugate vaccines for immunogenicity, including the induction of immunological memory, in the target age group before licensing. National regulatory authorities should ensure that the data made available to them are relevant to individual national immunization programmes, so that appropriate recommendations may be made regarding vaccine coadministration. For combinations of group C meningococcoccal conjugate vaccine and other antigens, either pre-combined or to be mixed immediately before injection, the national control authority should ensure that there are adequate studies to demonstrate that there is no clinically significant interference with the immunogenicity or induction of immunological memory by the meningococcal C conjugate component.

Two assays are utilized to measure immunogenicity of meningococcal C conjugate vaccines: the serum bactericidal antibody assay that is regarded as the gold standard and the serogroup C-specific IgG

enzyme-linked immunosorbent assay (ELISA). Early studies by Goldschneider *et al.* with polysaccharide vaccines (*2*) demonstrated that a serum bactericidal titre of 4 measured with human complement is an indicator of clinical protection against serogroup C meningococcal disease. The serum bactericidal antibody assay thus provides a good surrogate measurement of protective immunity associated with natural disease. Following the introduction of meningococcal group C conjugate vaccines in the United Kingdom, a re-evaluation of the correlates of protection for group C was performed (*3*) utilizing a large database of effectiveness data, the availability of sera for additional testing and serum bactericidal assays utilizing baby rabbit complement (*4*, *5*). Group C meningococci are more susceptible to the bactericidal activity of group C-specific antibodies when using baby rabbit complement than when using human complement, resulting in higher serum bactericidal assay titres for most specimens (*6*). Nevertheless, there is a general consensus that when baby rabbit serum is used as the source of complement, serum bactericidal assay titres of <8 are predictive of susceptibility to invasive meningococcal disease. From efficacy estimates in the UK and the proportion of responders in various clinical trials of meningococcal C conjugate vaccines, it has been demonstrated that a serum bactericidal assay titre of 8 is the appropriate cut-off correlating with short-term protection (*7*). This has now been supported by a group C seroprevalence study performed in the UK prior to the introduction of group C conjugate vaccines (*8*). Additional indicators may be used. These include:

— evidence of a fourfold or greater rise in serum bactericidal antibody titre between preimmunization and 1 month post-primary immunization sera;
— a serum bactericidal titre of ≥4 utilizing human complement (*3*).

The ELISA is an antigen-binding assay and is more reproducable than the serum bactericidal assay, which is an assay for functional antibodies (*9*). The ELISA can measure total or isotype-specific serum antibody responses and is thus a useful adjunct to the serum bactericidal assay. It is however crucial that the ELISA correlates with the serum bactericidal assay. A number of serogroup-C ELISAs have been shown to do this (*10–12*). Factors reported to increase the correlation include the use of highly purified polysaccharide, solid-phase derivatized polysaccharide antigens, and incorporation of chaotropic agents (thiocyanate) in the serum diluent.

Although the correlates for long-term protection are not currently known, antibody levels decline with time and immunological memory

may have to be relied upon. Immunization with meningococcal C conjugate vaccines primes for the ability to generate memory antibody responses upon subsequent exposure to plain meningococcal polysaccharide (*13*). Although unproven, the ability of an immunized person to generate a memory antibody response upon exposure to the pathogen may be an important second mechanism of protection, particularly when serum antibody concentrations are below the protective threshold. Recent data (*14*) demonstrate immunological memory in 4-years-old children who had been immunized with group C conjugate vaccine at 2, 3 and 4 months of age. At 4 years of age the antibody levels had decreased to prevaccination levels.

Laboratory correlates for the induction of immunological memory include

— demonstration of immunological memory by a serum bactericidal titre greater than or equal to that of the primary response 1 month following a 10 µg dose of plain polysaccharide administered at least 6 months after the primary series of immunization; or
— evidence of increase in avidity indices of serogroup C-specific IgG antibody 1–6 months after the primary series (*3*). Long-term monitoring will be necessary to determine whether induction of memory alone is enough to confer long-term protection against meningococcal disease

The serum antibody response to the carrier protein should also be measured in recipients of the meningococcal C conjugate vaccine to ensure that the conjugate vaccine does not interfere with protective immunity that is relevant to that protein. To date, carrier proteins such as diphtheria (CRM_{197}) and tetanus toxoids have been used in the conjugation of meningococcal C conjugate vaccines. Since some of carriers are also components of other vaccines administered to infants and children (e.g. diphtheria, tetanus, pertussis), antibody responses to those vaccines should be measured to ensure that there is no immune interference of clinical importance. The assay for these antibodies should be a bioassay or a validated equivalent.

The following reagents are available from the National Institute for Biological Standards and Control, Potters Bar, Herts., EN6 3QG, England, courtesy of the manufacturers and national regulatory agencies:

- Meningococcal group C polysaccharide, NIBSC code 98/730
- Meningococcal serogroup anticapsular antibody human ref. serum CDC1992, NIBSC code 99/706
- Methylated human serum albumin, NIBSC code 99/592

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