

5.3 Subjects

The test shall be performed on 12 to 15 healthy persons who have hands with healthy skin, without cuts or abrasions, and with short and clean fingernails. Although, in general, age is not a limiting factor, subjects should be at least 18 years of age.

5.4 Materials

5.4.1 Test organism

Escherichia coli K12 NCTC¹⁾ 10538.

NOTE : This test organism has been specifically chosen to meet health and safety guidance and ethical committee considerations. It is a K12 strain of *E. coli* of normal flora origin internationally recognized as being non-pathogenic. According to the UK catalogue of the National Collections of Industrial & Marine Bacteria (see [1] in Annex D), NCIMB strain 10083 is identical to NCTC 10538 and classified as a risk group 1 organism. The German Safety Ordinance on Gene Technology [2] also assigns the K12 strain to group 1. Directive 93/88/EEC [3] (Annex III to Directive 90/679/EEC [4]) explicitly states that non-pathogenic strains of *Escherichia coli* are *excluded* from the group 2 assignment.

5.4.2 Media and reagents

5.4.2.1 General

The reagents shall be of analytical grade and/or appropriate for microbiological purposes.

NOTE : To improve reproducibility, it is recommended that commercially available dehydrated material is used for the preparation of culture media. The manufacturer's instructions relating to the preparation of these products should be rigorously followed.

5.4.2.2 Water

The water shall be free from substances that are toxic or inhibiting to the bacteria. It shall be freshly glass distilled and not demineralised water.

Sterilize in the autoclave (see 5.5.2.1).

NOTE 1 : If the water is sterilized during the sterilization of the reagents, this is not necessary.

NOTE 2 : If distilled water of adequate quality is not available, water for injectable preparations (see European Pharmacopoeia) can be used.

¹⁾ NCTC 10538 is the collection number of a strain supplied by the National Collection of Type Cultures. This information is given for the convenience of users of this standard and does not constitute an endorsement by CEN of the product named. Corresponding strains supplied by another culture collection may be used if they can be shown to lead to the same results.