the vaccine is not acceptable if:

$$X_{\text{test}} - X_{\text{ref}} > C_1$$

the vaccine may be retested once if:

$$C_1 < X_{\text{test}} - X_{\text{ref}} < C_2$$

If the vaccine is retested, the means of the lesion scores for the vaccine to be tested and the reference vaccine are recalculated. The vaccine is not acceptable if:

$$\frac{X_{(\text{test 1}+\text{test 2})} - X_{(\text{ref 1}+\text{ref 2})}}{2} > C_3$$

The constants C_1, C_2 and C_3 are calculated from the expressions:

$$C_1 = 2.3\sqrt{\frac{2s^2}{N_1}}$$

$$C_2 = 2.6\sqrt{\frac{2s^2}{N_1}}$$

$$C_3 = 1.6\sqrt{\frac{2s^2}{N_1}}$$

N_1 = number of positive monkeys per vaccine test,

N_2 = number of positive monkeys in the two tests,

2.3 = normal deviate at the 1 per cent level,

2.6 = normal deviate at the 0.5 per cent level,

1.6 = normal deviate at the 5 per cent level.

A neurovirulence test in which the mean lesion score for the reference (X_{ref}) is not compatible with previous experience is not used for assessing a test vaccine. If the test is valid, the mean lesion score for the vaccine to be tested (X_{test}) is calculated and compared with that of the homotypic reference vaccine.

01/2008:20620

2.6.20. ANTI-A AND ANTI-B HAEMAGGLUTININS (INDIRECT METHOD)

Prepare in duplicate serial dilutions of the preparation to be examined in a 9 g/l solution of *sodium chloride R*. To each dilution of one series add an equal volume of a 5 per cent *V/V* suspension of group A_1 red blood cells previously washed three times with the sodium chloride solution. To each dilution of the other series add an equal volume of a 5 per cent *V/V* suspension of group B red blood cells previously washed three times with the sodium chloride solution. Incubate the suspensions at 37 °C for 30 min then wash the cells three times with the sodium chloride solution. Leave the cells in contact with a polyvalent anti-human globulin reagent for 30 min. Without centrifuging, examine each suspension for agglutination under a microscope.

01/2008:20621

2.6.21. NUCLEIC ACID AMPLIFICATION TECHNIQUES

1. INTRODUCTION

Nucleic acid amplification techniques are based on 2 different approaches:

1. amplification of a target nucleic acid sequence using, for example, polymerase chain reaction (PCR), ligase chain reaction (LCR), or isothermal ribonucleic acid (RNA) amplification,
2. amplification of a hybridisation signal using, for example, for deoxyribonucleic acid (DNA), the branched DNA (bDNA) method. In this case signal amplification is achieved without subjecting the nucleic acid to repetitive cycles of amplification.

In this general chapter, the PCR method is described as the reference technique. Alternative methods may be used, if they comply with the quality requirements described below.

2. SCOPE

This section establishes the requirements for sample preparation, *in vitro* amplification of DNA sequences and detection of the specific PCR product. With the aid of PCR, defined DNA sequences can be detected. RNA sequences can also be detected following reverse transcription of the RNA to complementary DNA (cDNA) and subsequent amplification.

3. PRINCIPLE OF THE METHOD

PCR is a procedure that allows specific *in vitro* amplification of segments of DNA or of RNA after reverse transcription into cDNA.

Following denaturation of double-stranded DNA into single-stranded DNA, 2 synthetic oligonucleotide primers of opposite polarity, anneal to their respective complementary sequences in the DNA to be amplified. The short double-stranded regions which form as a result of specific base pairing between the primers and the complementary DNA sequence, border the DNA segment to be amplified and serve as starting positions for *in vitro* DNA synthesis by means of a heat-stable DNA polymerase.

Amplification of the DNA occurs in cycles consisting of:

- heat denaturation of the nucleic acid (target sequence) into 2 single strands;
- specific annealing of the primers to the target sequence under suitable reaction conditions;
- extension of the primers, which are bound to both single strands, by DNA polymerase at a suitable temperature (DNA synthesis).

Repeated cycles of heat denaturation, primer annealing and DNA synthesis results in an exponential amplification of the DNA segment limited by the primers.

The specific PCR product known as an amplicon can be detected by a variety of methods of appropriate specificity and sensitivity.

Multiplex PCR assays use several primer pairs designed for simultaneous amplification of different targets in one reaction.

4. TEST MATERIAL

Because of the high sensitivity of PCR, the samples must be protected against external contamination with target sequences. Sampling, storage and transport of the test material are performed under conditions that minimise degradation of the target sequence. In the case of RNA target sequences, special precautions are necessary since RNA is