Problems with robustness may also arise with methods which use an initial ultracentrifugation step prior to extraction of the viral RNA. Therefore, to test the robustness of such methods, at least 20 plasma pools containing varying levels of HCV RNA, but lacking HCV specific antibodies, should be tested and found positive.

Cross contamination prevention should be demonstrated by the accurate detection of a panel of at least 20 samples consisting of alternate samples of negative plasma pools and negative plasma pools spiked with high concentrations of HCV (at least 10^2 times the 95 per cent cut-off value or at least 10^4 IU/ml).

Human plasma pools for NAT validation BRP are suitable for use as a negative control.

5. QUALITY ASSURANCE

For biological tests such as NAT, specific problems may arise which may influence both the validation and interpretation of results. The test procedures must be described precisely in the form of standard operating procedures (SOPs). These should cover:

- the mode of sampling (type of container, etc.),
- the preparation of mini-pools (where appropriate),
- the conditions of storage before analysis,
- the exact description of the test conditions, including precautions taken to prevent cross contamination or destruction of the viral RNA, reagents and reference preparations used,
- the exact description of the apparatus used,
- the detailed formulae for calculation of results, including statistical evaluation.

The use of a suitable run control (for example, an appropriate dilution of *hepatitis C virus RNA for NAT testing BRP* or plasma spiked with an HCV sample calibrated against the WHO HCV International Standard 96/790) can be considered a satisfactory system suitability check and ensures that the reliability of the analytical procedure is maintained whenever used.

Technical qualification: an appropriate installation and operation qualification programme should be implemented for each critical piece of the equipment used. Confirmation of analytical procedure performance after change of critical equipment (e.g. thermocyclers) should be documented by conducting a parallel test on 8 replicate samples of a plasma pool spiked with HCV RNA to a final concentration of 3 times the previously determined 95 per cent cut-off value. All results should be positive.

Operator qualification: an appropriate qualification programme should be implemented for each operator involved in the testing. To confirm successful training each operator should test at least 8 replicate samples of a plasma pool spiked with HCV RNA to a final concentration of 3 times the previously determined 95 per cent cut-off value. This test (8 replicate samples) should be repeated twice on two separate days, i.e. a total of 24 tests performed on three different days. All results should be positive.

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2.6.22. ACTIVATED COAGULATION FACTORS

Where applicable, determine the amount of heparin present (*2.7.12*) and neutralise the heparin by addition of *protamine sulphate R* (10 µg of protamine sulphate neutralises 1 IU of heparin). Prepare 1 to 10 and 1 to 100 dilutions of the preparation to be examined using *tris(hydroxymethyl)aminomethane buffer solution pH 7.5 R*. Place a series of polystyrene tubes in a water-bath at 37 °C and add to each tube 0.1 ml of *platelet-poor plasma R* and 0.1 ml of a suitable dilution of *cephalin R* or *platelet substitute R*. Allow to stand for 60 s. Add to each tube either 0.1 ml of one of the dilutions or 0.1 ml of the buffer solution (control tube). To each tube add immediately 0.1 ml of a 3.7 g/l solution of *calcium chloride R* (previously warmed to 37 °C) and measure, within 30 min of the original dilution, the time that elapses between addition of the calcium chloride solution and the formation of a clot. The test is not valid unless the coagulation time measured for the control tube is 200 s to 350 s.

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2.6.24. AVIAN VIRAL VACCINES: TESTS FOR EXTRANEOUS AGENTS IN SEED LOTS

GENERAL PROVISIONS

a) In the following tests, chickens and/or chicken material such as eggs and cell cultures shall be derived from chicken flocks free from specified pathogens (SPF) (*5.2.2*).

b) Cell cultures for the testing of extraneous agents comply with the requirements for the master cell seed of chapter *5.2.4. Cell cultures for the production of veterinary vaccines*, with the exception of the karyotype test and the tumorigenicity test, which do not have to be carried out.

c) In tests using cell cultures, precise specifications are given for the number of replicates, monolayer surface areas and minimum survival rate of the cultures. Alternative numbers of replicates and cell surface areas are possible as well, provided that a minimum of 2 replicates are used, the total surface area and the total volume of test substance applied are not less than that prescribed here and the survival rate requirements are adapted accordingly.

d) For a freeze-dried preparation, reconstitute using a suitable liquid. Unless otherwise stated or justified, the test substance must contain a quantity of virus equivalent to at least 10 doses of vaccine in 0.1 ml of inoculum.

e) If the virus of the seed lot would interfere with the conduct and sensitivity of the test, neutralise the virus in the preparation with a monospecific antiserum.

f) Monospecific antiserum and serum of avian origin used for cell culture or any other purpose, in any of these tests, shall be free of antibodies against and free from inhibitory effects on the organisms listed hereafter under 7. Antibody specifications for sera used in extraneous agents testing.

g) Where specified in a monograph or otherwise justified, if neutralisation of the virus of the seed lot is required but difficult to achieve, the *in vitro* tests described below are adapted, as required, to provide the necessary guarantees of freedom from contamination with an extraneous agent.

h) Other types of tests than those indicated may be used provided they are at least as sensitive as those indicated and of appropriate specificity. Nucleic acid amplification techniques (*2.6.21*) give specific detection for many agents and can be used after validation for sensitivity and specificity.

1. TEST FOR EXTRANEOUS AGENTS USING EMBRYONATED HENS' EGGS

Use a test substance, diluted if necessary, containing a quantity of neutralised virus equivalent to at least 10 doses of vaccine in 0.2 ml of inoculum. Suitable antibiotics may be added. Inoculate the test substance into 3 groups of 10 embryonated hens' eggs as follows: