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2.5.14. CALCIUM IN ADSORBED VACCINES

All solutions used for this test must be prepared using water R.

Determine the calcium by atomic emission spectrometry (*2.2.22, Method I*). Homogenise the preparation to be examined. To 1.0 ml add 0.2 ml of *dilute hydrochloric acid R* and dilute to 3.0 ml with *water R*. Measure the absorbance at 620 nm.

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2.5.15. PHENOL IN IMMUNOSERA AND VACCINES

Homogenise the preparation to be examined. Dilute an appropriate volume with *water R* so as to obtain a solution presumed to contain 15 µg of phenol per millilitre. Prepare a series of reference solutions with *phenol R* containing 5 µg, 10 µg, 15 µg, 20 µg and 30 µg of phenol per millilitre respectively. To 5 ml of the solution to be examined and to 5 ml of each of the reference solutions respectively, add 5 ml of *buffer solution pH 9.0 R*, 5 ml of *aminopyrazolone solution R* and 5 ml of *potassium ferricyanide solution R*. Allow to stand for 10 min and measure the intensity of colour at 546 nm.

Plot the calibration curve and calculate the phenol content of the preparation to be examined.

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2.5.16. PROTEIN IN POLYSACCHARIDE VACCINES

Test solution. Use a volumetric flask with a suitable volume for preparation of a solution containing about 5 mg per millilitre of dry polysaccharide. Transfer the contents of a container quantitatively to the flask and dilute to volume with *water R*. Place 1 ml of the solution in a glass tube and add 0.15 ml of a 400 g/l solution of *trichloroacetic acid R*. Shake, allow to stand for 15 min, centrifuge for 10 min at 5000 r/min and discard the supernatant. Add 0.4 ml of *0.1 M sodium hydroxide* to the centrifugation residue.

Reference solutions. Dissolve 0.100 g of *bovine albumin R* in 100 ml of *0.1 M sodium hydroxide* (stock solution containing 1 g of protein per litre). Dilute 1 ml of the stock solution to 20 ml with *0.1 M sodium hydroxide* (working dilution 1: 50 mg of protein per litre). Dilute 1 ml of the stock solution to 4 ml with *0.1 M sodium hydroxide* (working dilution 2: 250 mg of protein per litre). Place in 6 glass tubes 0.10 ml, 0.20 ml and 0.40 ml of working dilution 1 and 0.15 ml, 0.20 ml and 0.25 ml of working dilution 2. Make up the volume in each tube to 0.40 ml using *0.1 M sodium hydroxide*.

Prepare a blank using 0.40 ml of *0.1 M sodium hydroxide*.

Add 2 ml of *cupri-tartaric solution R3* to each tube, shake and allow to stand for 10 min. Add to each tube 0.2 ml of a mixture of equal volumes of *phosphomolybdotungstic reagent R* and *water R*, prepared immediately before use. Stopper the tubes, mix by inverting and allow to stand in the dark for 30 min. The blue colour is stable for 60 min. If necessary, centrifuge to obtain clear solutions.

Measure the absorbance (*2.2.25*) of each solution at 760 nm using the blank as the compensation liquid. Draw a calibration curve from the absorbances of the 6 reference solutions and the corresponding protein contents and read from the curve the content of protein in the test solution.

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2.5.17. NUCLEIC ACIDS IN POLYSACCHARIDE VACCINES

Test solution. Use a volumetric flask with a suitable volume for preparation of a solution containing about 5 mg per millilitre of dry polysaccharide. Transfer the contents of a container quantitatively to the flask and dilute to volume with *water R*.

Dilute the test solution if necessary to obtain an absorbance value suitable for the instrument used. Measure the absorbance (*2.2.25*) at 260 nm using *water R* as the compensation liquid.

The absorbance of a 1 g/l solution of nucleic acid at 260 nm is 20.

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2.5.18. PHOSPHORUS IN POLYSACCHARIDE VACCINES

Test solution. Use a volumetric flask with a suitable volume for preparation of a solution containing about 5 mg per millilitre of dry polysaccharide. Transfer the contents of a container quantitatively to the flask and dilute to volume with *water R*. Dilute the solution so that the volume used in the test (1 ml) contains about 6 µg of phosphorus. Transfer 1.0 ml of the solution to a 10 ml ignition tube.

Reference solutions. Dissolve 0.2194 g of *potassium dihydrogen phosphate R* in 500 ml of *water R* to give a solution containing the equivalent of 0.1 mg of phosphorus per millilitre. Dilute 5.0 ml of the solution to 100.0 ml with *water R*. Introduce 0.5 ml, 1.0 ml and 2.0 ml of the dilute solution into 3 ignition tubes.

Prepare a blank solution using 2.0 ml of *water R* in an ignition tube.

To all the tubes add 0.2 ml of *sulphuric acid R* and heat in an oil bath at 120 °C for 1 h and then at 160 °C until white fumes appear (about 1 h). Add 0.1 ml of *perchloric acid R* and heat at 160 °C until the solution is decolorised (about 90 min). Cool and add to each tube 4 ml of *water R* and 4 ml of *ammonium molybdate reagent R*. Heat in a water-bath at 37 °C for 90 min and cool. Adjust the volume to 10.0 ml with *water R*. The blue colour is stable for several hours.

Measure the absorbance (*2.2.25*) of each solution at 820 nm using the blank solution as the compensation liquid. Draw a calibration curve with the absorbances of the 3 reference solutions as a function of the quantity of phosphorus in the solutions and read from the curve the quantity of phosphorus in the test solution.