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2.5.22. URONIC 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 solution so that the volumes used in the test contain 4 µg to 40 µg of glucuronic acid (uronic acids). Introduce 0.25 ml, 0.50 ml and 1.0 ml of the diluted solution into 3 tubes.

Reference solutions. Dissolve 50 mg of *sodium glucuronate R* in 100 ml of *water R* (stock solution containing 0.4 g of glucuronic acid per litre). Immediately before use, dilute 5 ml of the stock solution to 50 ml with *water R* (working dilution: 40 mg of glucuronic acid per litre). Introduce 0.10 ml, 0.25 ml, 0.50 ml, 0.75 ml, and 1.0 ml of the working dilution into 5 tubes.

Prepare a blank using 1 ml of *water R*.

Make up the volume in each tube to 1 ml with *water R*. Place the tubes in iced water and add dropwise and with continuous stirring to each tube 5.0 ml of *borate solution R*. Stopper the tubes and place in a water-bath for 15 min. Cool to room temperature. Add 0.20 ml of a 1.25 g/l solution of *carbazole R* in *ethanol R* to each tube. Stopper the tubes and place in a water-bath for 15 min. Cool to room temperature. Measure the absorbance (*2.2.25*) of each solution at 530 nm using the blank as the compensation liquid.

Draw a calibration curve from the absorbances for the 5 reference solutions and the corresponding content of glucuronic acid and read from the curve the quantity of glucuronic acid in the test solution for each volume tested. Calculate the mean of the 3 values.

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2.5.23. SIALIC ACID IN POLYSACCHARIDE VACCINES

Test solution. Transfer quantitatively the contents of one or several containers to a volumetric flask of a suitable volume that will give a solution with a known concentration of about 250 µg per millilitre of polysaccharide and dilute to volume with *water R*. Using a syringe, transfer 4.0 ml of this solution to a 10 ml ultrafiltration cell suitable for the passage of molecules of relative molecular mass less than 50 000. Rinse the syringe twice with *water R* and transfer the rinsings to the ultrafiltration cell. Carry out the ultrafiltration, with constant stirring, under *nitrogen R* at a pressure of about 150 kPa. Refill the cell with *water R* each time the volume of liquid in it has decreased to 1 ml and continue until 200 ml has been filtered and the remaining volume in the cell is about 2 ml. Using a syringe, transfer this residual liquid to a 10 ml volumetric flask. Wash the cell with 3 quantities, each of 2 ml, of *water R*, transfer the washings to the flask and dilute to 10.0 ml with *water R* (test solution). In each of 2 test-tubes place 2.0 ml of the test solution.

Reference solutions. Use the reference solutions prescribed in the monograph.

Prepare 2 series of 3 test-tubes, place in the tubes of each series 0.5 ml, 1.0 ml and 1.5 ml respectively, of the reference solution corresponding to the type of vaccine to be examined and adjust the volume in each tube to 2.0 ml with *water R*.

Prepare blank solutions using 2.0 ml of *water R* in each of 2 test-tubes.

To all the tubes add 5.0 ml of *resorcinol reagent R*. Heat at 105 °C for 15 min, cool in cold water and transfer the tubes to a bath of iced water. To each tube add 5 ml of *isoamyl alcohol R* and mix thoroughly. Place in the bath of iced water for 15 min. Centrifuge the tubes and keep them in the bath of iced water until the examination by absorption spectrophotometry. Measure the absorbance (*2.2.25*) of each supernatant solution at 580 nm and 450 nm using *isoamyl alcohol R* as the compensation liquid. For each wavelength, calculate the absorbance as the mean of the values obtained with 2 identical solutions. Subtract the mean value for the blank solution from the mean values obtained for the other solutions.

Draw a graph showing the difference between the absorbances at 580 nm and 450 nm of the reference solutions as a function of the content of *N*-acetylneuraminic acid and read from the graph the quantity of *N*-acetylneuraminic acid (sialic acid) in the test solution.

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2.5.24. CARBON DIOXIDE IN GASES

Carbon dioxide in gases is determined using an infrared analyser (see Figure 2.5.24.-1).

The infrared analyser comprises 2 generators of identical infrared beams. The generators are equipped with reflectors and coils electrically heated to low red heat. One beam crosses a sample cell and the other beam crosses a reference cell. The sample cell receives a stream of the gas to be analysed and the reference cell contains *nitrogen R1*. The 2 chambers of the detector are filled with *carbon dioxide R1* and the radiation is automatically received selectively. The absorption of this radiation produces heat and differential expansion of the gas in the 2 chambers, owing to absorption of some of the emitted radiation by the carbon dioxide in the gas to be examined. The pressure difference between the 2 chambers of the detector causes distension of the metal diaphragm that separates them. This diaphragm is part of a capacitor, whose capacitance varies with the pressure difference, which itself depends on the carbon dioxide content in the gas to be examined. Since the infrared beams are periodically blocked by a rotating chopper, the electric signal is frequency modulated.

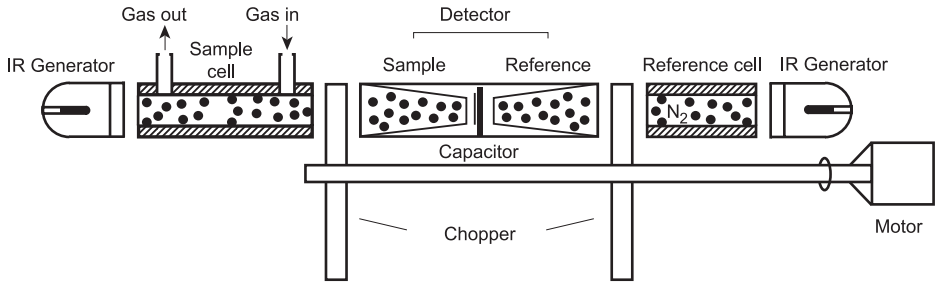

Figure 2.5.24.-1. – *Infrared analyser*