Fibrin sealant kit

DEFINITION
Fibrin sealant kit is essentially composed of 2 components, namely fibrinogen concentrate (component 1), a protein fraction containing human fibrinogen and a preparation containing human thrombin (component 2). A fibrin clot is rapidly formed when the 2 thawed or reconstituted components are mixed. Other ingredients (for example, human coagulation factor XIII, a fibrinolysis inhibitor or calcium ions) and stabilisers (for example, Human albumin solution (0255)) may be added. No antimicrobial preservative is added.

Human constituents are obtained from plasma that complies with the requirements of the monograph on Human plasma for fractionation (0853). No antibiotic is added to the plasma used.

When thawed or reconstituted as stated on the label, component 1 contains not less than 40 g/l of clottable protein; the thrombin activity of component 2 varies over a wide range (approximately 4-1000 IU/ml).

PRODUCTION
The method of preparation includes a step or steps that have been shown to remove or to inactivate known agents of infection; if substances are used for inactivation of viruses during production, the subsequent purification procedure must be validated to demonstrate that the concentration of these substances is reduced to a suitable level and any residues are such as not to compromise the safety of the preparation for patients.

Constituents or mixtures of constituents are passed through a bacteria-retentive filter and distributed aseptically into sterile containers. Containers of freeze-dried constituents are closed under vacuum or filled with oxygen-free nitrogen or other suitable inert gas before being closed. In either case, they are closed so as to exclude micro-organisms.

If the human coagulation factor XIII content in component 1 is greater than 10 units/ml, the assay of factor XIII is carried out.

CHARACTERS
Freeze-dried constituents are hygroscopic, white or pale yellow powders or friable solids. Frozen constituents are colourless or pale yellow, opaque solids. Liquid constituents are colourless or pale yellow.

For the freeze-dried or frozen constituents, reconstitute or thaw as stated on the label immediately before carrying out the identification and the tests, except those for solubility and water.

Component 1 (fibrinogen concentrate)

IDENTIFICATION
A. It complies with the limits of the assay of fibrinogen.
B. It complies with the limits of the assay of factor XIII (where applicable).

TESTS
pH (2.2.3): 6.5 to 8.0.

Solubility. Freeze-dried concentrates dissolve within 20 min in the volume of solvent for reconstitution and at the temperature stated on the label, forming an almost colourless, clear or slightly turbid solution.

Stability of solution. No gel formation appears at room temperature during 120 min following thawing or reconstitution.

Water. Determined by a suitable method, such as the semi-microdetermination (2.5.12), loss on drying (2.2.32) or near infrared spectrophotometry (2.2.40), the water content is within the limits approved by the competent authority.

Sterility (2.6.1). It complies with the test for sterility.
ASSAY

**Fibrinogen (clottable protein).** The estimated content in milligrams of clottable protein is not less than 70 per cent and not more than 130 per cent of the content stated on the label.

Mix 0.2 ml of the reconstituted preparation with 2 ml of a suitable buffer solution (pH 6.6-7.4) containing sufficient human thrombin R (approximately 3 IU/ml) and calcium (0.05 mol/l). Maintain at 37 °C for 20 min, separate the precipitate by centrifugation (5000 g, 20 min), wash thoroughly with a 9 g/l solution of sodium chloride R and determine the protein as nitrogen by sulphuric acid digestion (2.5.9). Calculate the protein content by multiplying the result by 8.0. If for a particular preparation this method cannot be applied, use another validated method for determination of fibrinogen.

**Factor XIII.** Where the label indicates that the human coagulation factor XIII activity is greater than 10 units/ml, the estimated activity is not less than 80 per cent and not more than 120 per cent of the activity stated on the label.

Make at least 3 suitable dilutions of thawed or reconstituted component 1 and of human normal plasma (reference preparation) using as diluent coagulation factor XIII deficient plasma or another suitable diluent. Add to each dilution suitable amounts of the following reagents:

- activator reagent, containing bovine or human thrombin, a suitable buffer, calcium chloride and a suitable inhibitor such as Gly-Pro-Arg-Pro-Ala-NH₂, which inhibits clotting of the sample but does not prevent coagulation factor XIII activation by thrombin;

- detection reagent, containing a suitable factor XIII-specific peptide substrate, such as Leu-Gly-Pro-Gly-Ser-Lys-Ile-Leu-Gly-NH₂ and glycine ethyl ester as 2nd substrate in a suitable buffer solution,

- NADH reagent, containing glutamate dehydrogenase, α-ketoglutarate and NADH in a suitable buffer solution.

After mixing, the absorbance changes (ΔA/min) are measured at a wavelength of 340 nm, after the linear phase of the reaction is reached.

1 unit of factor XIII is equal to the activity of 1 ml of human normal plasma.

Calculate the activity of the test preparation by the usual statistical methods (5.3, for example). The confidence limits (P = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated activity.

**Component 2 (thrombin preparation)**

**IDENTIFICATION**

It complies with the limits of the assay of thrombin.

**TESTS**

**pH (2.2.3):** 5.0 to 8.0.

**Solubility.** Freeze-dried preparations dissolve within 5 min in the volume of solvent for reconstitution stated on the label, forming a colourless, clear or slightly turbid solution.

**Water.** Determined by a suitable method, such as the semi-microdetermination (2.5.12), loss on drying (2.2.32) or near infrared spectrophotometry (2.2.40), the water content is within the limits approved by the competent authority.

**Sterility (2.6.1).** It complies with the test for sterility.

**ASSAY**

**Thrombin.** The estimated activity is not less than 80 per cent and not more than 125 per cent of the activity stated on the label.

If necessary, dilute the reconstituted preparation to be examined to approximately 2-20 IU of thrombin per millilitre using as diluent a suitable buffer pH 7.3-7.5, such as imidazole buffer solution pH 7.3 R containing 10 g/l of human albumin R or bovine albumin R. To a suitable volume of the dilution, add a suitable volume of fibrinogen solution (1 g/l of clottable protein) warmed to 37 °C and start measurement of the clotting time immediately. Repeat the procedure with each of at least 3 dilutions, in the range stated above, of a reference preparation of thrombin, calibrated in International Units. Calculate the activity of the test preparation by the usual statistical methods (5.3, for example). The confidence limits (P = 0.95) are not less than 80 per cent and not more than 125 per cent of the estimated activity.

**STORAGE**

Protected from light and, for freeze-dried components, in an airtight container.

**LABELLING**

The label states:

- the amount of fibrinogen (milligrams of clottable protein), thrombin (International Units) per container, and coagulation factor XIII, if this is greater than 10 units/ml,
- where applicable, the name and volume of solvent to be used to reconstitute the components.

FINASTERIDE

**Finasteridum**

C₂₃H₃₆N₂O₂

M, 372.6

98319-26-7

**DEFINITION**

N(1,1-Dimethylethyl)-3-oxo-4-aza-5α-androst-1-ene-17β-carboxamide.

**Content:** 98.0 per cent to 102.0 per cent (dried substance).

**CHARACTERS**

**Appearance:** white or almost white, crystalline powder.

**Solubility:** practically insoluble in water, freely soluble in ethanol and in methylene chloride.

It shows polymorphism (5.9).

**IDENTIFICATION**

Infrared absorption spectrophotometry (2.2.24).

Comparison: finasteride CRS.