Question 1:

Which protein used as a marker in IFM can be altered in dystrophic EB?

- Collagen type IV
- Collagen type XVII
- Collagen type VII

*Explanation:*

Mutations in the gene encoding collagen VII are the cause of dystrophic EB. In most cases, the immunoreactivity for collagen VII is reduced or lost in the skin of the patients and represents a good prognostic marker for disease severity.

- Laminin 332
- None of the above

Question 2:

Which of the following is not true regarding IFM?

- It may indicate the skin layer where cleavage occurs.
- It may indicate the mutated gene and dysfunctional protein in EB.
- It may indicate the presence of revertant mosaicism in the skin of an EB patient.
- The specimen may contain areas with artificial cleavage.
- It always yields specific and clear results.

*Explanation:*
IFM does not always yield specific and clear results. Technical issues may induce artefacts, while mild phenotypes may demonstrate no specific features.

**Question 3:**

Which is the best biopsy site if EB is suspected and use of IFM is requested?

- Near a recent blister (less than 12 hours).
- An erosive area.
- The skin should be rubbed with an eraser to induce new blister formation, and a biopsy of that site should be taken several minutes later.

**Explanation:**

Determination of the level of skin cleavage is the major information provided by IFM and is required for EB classification. Non-blistered skin or old blisters may deliver false data or be uninformative.

- Palms or soles.
- Any blister will indicate the layer where skin cleavage occurs.

**Question 4:**

Which of the following statements is true in junctional EB?

- In a junctional blister, collagen IV stains at the blister floor.
- Immunoreactivity for collagen VII is altered.
- Immunoreactivity for collagen XVII may be altered.

**Explanation:**

IFM discriminates between junctional and dermal cleavage based on the relative position of the immunoreactivity for collagen IV, a main constituent of the epidermal...
basement membrane. Reduced or absent immunoreactivity for collagen XVII indicates the presence of COL17A1 mutations and signifies intermediate junctional EB.

Collagen VII stains at the blister roof.

The level of cleavage can be assessed by Haematoxylin eosin staining.

Question 5:

Which of the following is wrong regarding the primary antibodies used in IFM?

A minimal panel of antibodies can be employed.

For an extended IFM, antibody costs are significantly high.

Primary antibodies are always conjugated with a fluorescent compound.

*Explanation:*

Primary antibodies are usually not associated with a fluorescent compound; detection requires the use of fluorescent conjugated secondary antibodies.

A negative control (secondary antibodies without primary antibodies) should always be run.

An extended panel of primary antibodies can be used depending on the complexity of the question.