CISH on Cytology Specimens using the Zyto*Dot* 2C Method:

Material and reagents needed from ZytoVision:

- Zyto*Dot* 2C CISH Implementation Kit [C-3044-40]
- Cytology Pepsin Solution (ES2) [ES-0002-4]
- Formaldehyde Dilution Buffer Set [PT-0006-100]

Preparatory steps:

Day 1:

- *Preparation of ethanol series (70%, 90% and 100% ethanol solutions):* Dilute 7, 9 and 10 parts of 100% ethanol with 3, 1 and 0 parts of dH_2O. These solutions can be stored in suitable containers and be reused.

- *Prepare 1x Wash Buffer TBS :* Dilute 1 part 20x Wash Buffer TBS **[WB5]** with 19 parts dH_2O. Diluted Wash 1x Wash Buffer TBS lasts for one week when stored at 2-8°C.

- *Prepare 1% Formaldehyde solution:* For 100 ml 1% Formaldehyde solution mix either 2.7 ml of 37% neutrally buffered formaldehyde or 10 ml of 10% neutrally buffered formaldehyde with 10 ml of 10x $MgCl_2$ **[PT4]** and 10 ml of 10x PBS **[PT5]** and adjust volume to 100 ml with dH_2O. Mix thoroughly.

- *Prepare 3% H_2O_2:* Dilute 1 part of 30% H_2O_2 with 9 parts of 100% methanol.

- *ZytoDot 2C CISH Probe:* Bring to RT before use.

Pretreatment (Proteolysis/Post-Fixation) [Day 1]:

- Apply dropwise Cytology Pepsin Solution **[ES2]** (4-8°C) to the cytology specimen and incubate at 37°C in a humidity chamber.
  Depending on multiple factors, e.g. nature and duration of fixing as well as nature of cells, different incubation times may be required. We recommend an Incubation time of 5-15 min for cytology specimens. As a general rule, we recommend to ascertain the optimum time for proteolysis in pre-tests.

- Incubate slides for 5 min in 1x Wash Buffer TBS.

- Incubate slides for 5 min in 1% Formaldehyde solution.

- Incubate slides for 5 min in 1x Wash Buffer TBS.

- Dip in water.

- Incubate slides for 5 min in 3% H_2O_2.

- Wash 2x 1 min in dH_2O.

- Dehydration in 70%, 90% and 100% ethanol for 1 min each.

- Air dry specimens.

Denaturation and Hybridization [Day 1]:

- Pipette 10 µl Zyto*Dot* 2C probe onto each sample.
  A gentle warming of the probe, as well as using a pipette tip which has been cut to increase the size of the opening, can make the pipetting process easier.

- Avoiding trapped bubbles, cover the samples with a coverslip. Seal the coverslip e.g. with a layer of hot glue from an adhesive pistol or with rubber cement.

- Denature the slides at 72 ± 1°C for 2 min e.g. on a hot plate.

- Transfer the slide to a humidity chamber and hybridize overnight at 37°C e.g. in a hybridization oven.

From here on the instructions on the second day protocol of the Zyto*Dot* 2C CISH-Implementation Kit [C-3044-40] can be followed.