Vision*Array* Practical Procedure – Technical Tips and Tricks:

Technical tips & tricks which should be considered before performing the Vision*Array* method:

DNA Extraction:

- What kind of raw material can be used?
  - HPV: FFPE tissue samples, cervical swabs/brush specimen, ThinPrep
  - MYCO: FFPE tissue samples, pulmonary smears, cultivated samples

- Which DNA extraction kits are available?
  FFPE samples: Vision*Array* FFPE DNA Extraction Kit.
  Cytology samples: Vision*Array* Cytology DNA Extraction Kit.

- Which DNA concentration is recommended?
  After extraction a measurement of the DNA concentration is necessary in order to check the quality and quantity of the DNA. Each sample should have a DNA concentration of at least 15 ng/µl with a high degree of purity (260/280: ~1.8). For higher concentrated DNA it is not necessary to dilute the sample before performing a PCR.

- How to avoid DNA contaminations?
  When using a microtome the tissue sections should be placed immediately in a reaction tube after cutting. The microtome blade should be changed between different tissue samples. The same applies for already fixated tissue samples mounted on glass slides. The scraper should be changed between different samples.

PCR:

- How to avoid DNA contaminations?
  Separate rooms for working steps with and without DNA as well as using clean benches are necessary for preparation of the PCR to avoid contaminations.

- Which DNA Polymerase should be used for the PCR?
  The usage of the Vision*Array* HPV PreCise Master Mix or Vision*Array* MYCO PreCise Master Mix 2.0 is absolutely mandatory.
  The ready-to-use Master Mixes enables a more fail-safe and convenient implementation plus less hands-on time for preparation of the Vision*Array* PCR. The Master Mixes contain all components for the PCR with exception of the DNA sample.

- Which DNA sample volume is needed for a successful Vision*Array* PCR?
  For the PCR assays 2.5-5 µl DNA sample is fully sufficient. The use of increased volumes (10 µl) may lead to an inhibition of the PCR due to the EDTA contained in many elution buffers of the extraction kits or due to other inhibiting components contained in the DNA sample.

- Which are the appropriate storage conditions of the PCR products?
  The storage of the PCR product has a high impact on the product quality and on the signal quality after detection. For long term storage the PCR products should be stored frozen at -16…-22°C to prevent degradation due to UDG residual activity. We tested this residual UDG activity and storage for one day at room temperature led to a significantly decreased signal intensity after the detection procedure. Thus, the storage of the PCR product at room temperature should be as short as possible.

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Hybridization/Detection:

- How is the Hybridization Mix applied?
  The hybridization mix should carefully be applied on the left side of the chip field (with chip label on the right) avoiding air bubble formation. The solution is then distributed on the chip with the help of the supplied plastic lid: Position the lower edge of the lid on the left hand side of the blue sticky chip frame and slowly lower the lid to the right, dispersing the hybridization mix across the chip without trapping air bubbles.

- Which steps should be performed without any break?
  The steps before and after hybridization should be performed quickly as an incubation at room temperature may lead to unspecific background staining. After preparation of the hybridization mix and its application on the chip, transfer the slides quickly into the hybridizer or hybridization oven. This step should be done for each chip one after the other, never in parallel. After hybridization, place the chips directly into the Wash Buffer. Slides should remain at hybridization temperature until they are handled. Exposure to room temperature should be as short as possible.

- Is there a limited number of chips used during the washing step?
  We recommend not to use more than six chips per staining jar and washing step.

- Why is it necessary to use a slide centrifuge for drying of the chips?
  After washing the usage of the slide centrifuge is absolutely mandatory in order to prevent droplets left on the chip. Any residual Wash Buffer leads to background staining on the chip and dilution of the subsequently applied solution. In order to prevent an air drying of the chips during the centrifugation process, take just two chips simultaneously out of the Wash Buffer for centrifugation. Additional chips should remain in the Wash Buffer in the meantime.

- Is there an impact on signal quality when performing the Vision*Array* procedure under a clean bench?
  Performing the hybridization/detection procedure under a working clean bench has a significant negative influence on the signal quality due to air movement and should thus be avoided.

Vision*Array* Chip Storage and Stability:

- What are the storage conditions for the Vision*Array* Chips? How long are they usable after opening?
  The Vision*Array* Chips should be stored at -16…-22°C. If these storage conditions are followed, the chips are stable, without loss of performance, at least until the expiry date printed on the label. After opening the original packaging, unused chips should be stored at -16…-22°C as well. Under these storage conditions the chips are usable for two months without loss of performance.