Frequently Asked Questions Microblot-Array (MBA)

TECHNOLOGY

- **What is the significance of the different color coding of the microtiter plates?**
  - IgA = green, IgG= blue, IgM = red
  - It is intended for an easier orientation in the plate, not to mix up the kits.

- **Does a calibration have to be performed regularly? If so, must this be done by the technician, or can the customer do it himself?**
  - Autodiagnostics is to be performed every 20 hrs of use (in reality this means every 3-6 months depending on how many plates they are running each week).
  - Validation by service team is to be performed once a year.

- **Is there a positive control and a calibrator that have to be carried along?**
  - Each well has test and conjugate control (in triplicate).
  - Each well has 4 calibrators (in triplicate).
  - There is positive control included in the kits.

- **What are calibrators and controls?**
  - **Calibrators:** these are different concentrations of conjugated anti-Hu antibody in a special solution. The concentrations are optimized so that the values of the individual calibrators reach the U/ml TC:1000 C1:700 C2:375 C3:75 C4:30. The created calibration curve allows:
    - Quantify the measured values for individual antigens
    - Correlate deviations due to the use of different devices or manual processing
    - Correlate deviations due to different physical conditions – temperature
    - Correlate deviations due to a decrease in the quality of solutions - conjugate, substrate
  - **Conjugate control:** It is immunoglobulin A, G or M in a special solution. Both calibrators and conjugate controls can be affected by sample composition. Values may be also affected by different membrane backgrounds. However, if the conjugate control is valid, everything should be OK. A drop to 500 U/ml is not a cause for concern, it is still quite high value.

- **What is the biggest difference between the blot, BlotLine, the BlueBlot line and the Microblot array?**
  - Intended use, composition of the kit, type of processing and evaluation
  - BLOT
    - **Intended use**: immunoblot for confirmation of ELISA results (infectious diseases and autoimmunity disorders)

        - **Kit composition**: 20 standard strips (native, whole cell antigens) + reagents bottles (Universal solution, conjugate, substrate, positive/negative controls)
        - **Processing**: both manual and automated (open immunoblot analyzer)
        - **Evaluation**: both visual and software (any office scanner can be used)
    - BLOT-LINE
        - **Intended use**: immunoblot for confirmation of ELISA results (infectious diseases and autoimmunity disorders)
        - **Kit composition**: 20 standard strips (with recombinant antigens) + reagents bottles (Universal solution, conjugate, substrate, positive/negative controls)
        - **Processing**: both manual and automated (open immunoblot analyzer)
        - **Evaluation**: both visual and software (any office scanner can be used)
    - BlueBLOT-LINE
        - **Intended use:** immunoblot for confirmation of ELISA results (infectious diseases)
        - **Kit composition:** 24 breakable strips in plastic cover (recombinant antigens) + 24 ready to use cartridges
        - **Processing**: fully automated solution intended to be performed only on the BlueDiver Instrument
        - **Evaluation**: only SW evaluation possible (special scanning device)
    - Microblot-Array
        - **Intended use:** MULTIPLEX method for both screening and confirmation (advanced panels because of the huge capacity compared to BLOT), for infectious diseases and autoimmunity disorders
        - **Kit composition:** 96/48 breakable wells (with recombinant antigens) + reagents bottles (Universal solution, conjugate, substrate, positive control)
        - **Processing**: both manual and automated (any open ELISA anaylser)
        - **Evaluation**: only SW evaluation possible (TestLine Reader)

SAMPLES

- **Which patient material can be used and how many µl/tests of which material is needed?**
    - Serum (10 µl), CSF (50 µl), synovial fluid (10 µl)

- **How many samples can be processed at once in analyzer?**
    - Depending on the automated device (each has a different number of plates/ assays/ samples it can run), one MBA kit contains 96 wells though (so with one kit up to 96 patients).

PROCESSING

- For which fully automated devices has the MBA method already been tested?

  - DS2 (2 plates), DSX (4 plates), Agility (12 plates) from Dynex (TestLine supply these instruments)
  - Gemini (2-3 plates) from Stratec (TestLine supply this instrument)
  - Triturus (4 plates) from Grifols (TestLine does not supply this one)

- **How many parameters can be processed simultaneously?**
  - Depending on the automatic analyser, usually up to 12
  - Gemini – 24 different parameters in one run
    - Number of plates: 2/2
    - Number of assays: 12assays/plate (8 assays/strip)
  - DS2 - 12 different parameters in one run
    - Number of plates: 1/2
    - Number of assays: 12 assays/plate (1 assay/strip)
  - DSX - 24 different Parameters in one run
    - Number of plates: 2/4
    - Number of assays: 12 assays/plate (1 assay/strip)
  - Agility - 8 different parameters in one run
    - Number of plates: 2/12
    - Number of assays: 4 assays/plate (1 assay/strip)

- **If the MBA is processed manually, is it still possible to use different parameters?**
  - Yes, processing of all the MBA methods is the same.

- **Can other fully automated ELISA processing devices also be used? What is the requirement?**
  - Yes, but after the validation of the assay and machine from the TestLine team with our known samples, to be discussed with the TL area sales manager, production, and service team.

- **How many samples can be processed at once in analyzer?**
  - Depending on the automated device (each has a different number of plates/ assays/ samples it can run), one MBA kit contains 96 wells though (so with one kit up to 96 patients).

- **How long does the fully automated processing take?**
  - It depends on the automated device or manual process. The method itself though takes around 2 hrs + 30-45 mins drying in TL dryer.

- **Why does the automatic processing require 2 extra bottles of Universal Solution?**
  - Because of the dead volume of wash bottles in automatic analyzers.

- **Can MBA and ELISA be processed simultaneously in one ELISA processing device?**

- MBA and ELISA can be processed simultaneously but it depends on capacity of the machine.
- It is not so recommended because the analysis time will increase significantly.
- You can combine them eg. on DSX, Agility or Gemini but not on DS2.

DRYING/TESTLINE DRYER

- **How long does it take to dry the plates and how long does it take in the TestLine dryer?**
  - Without the dryer probably around 2-3 hours.
  - It takes 30-45 minutes with the TestLine dryer.

- **Is it possible to dry the plates in an incubator?**
  - Technically it is possible although NOT recommended, since we supply the dryer which has the best drying conditions for the plates.

EVALUATION

- **How long can the plates be evaluated after processing, how long is the reaction stable?**
  - If the plate is stored in a clean, dark, and dry place, even a week after. It is possible but it is NOT recommended.

- **Can the plates be evaluated directly after processing?**
  - No, the plates need to be completely dry before the evaluation.

- **Are the results of the Microblot Array quantitative or qualitative**
  - The results are **quantitative**.

- **Why are the antigens contained in triplicate spots in the well? How many spots must be present for an evaluation to still take place?**
  - Antigens are in triplicate for more precise evaluation. The software calculates the median of these three spots.
  - If one of the three spots is damaged or there is any dirt, then the abnormal value is automatically excluded from the evaluation and the calculation is made from the two remaining ones.

- **What happens if the barcode on the well is damaged, can the evaluation still take place?**
  - If the QR code is damaged, you can choose the type of the test and lot manually, so the evaluation still takes place.

- **What happens if a spot in the well is damaged? What happens if a reference spot in the well is damaged?**
  - Depending on the spot. If it is an antigen spot and just one the evaluation still can take place.

  - If the reference spot is too damaged depending on the type of damage either you can position the grid manually or you have to repeat the test with a new well.

- **Can I use an ELISA reader to evaluate the microarray?**
  - No, you cannot.

- **Why are there Empty spots in the well?**
  - Because each kit has different number of antigens.

KITS

- **Which kits are currently available?**
  - **Autoimmunity**: ANA, Liver profile (validaton kit)
  - **Infectious serology**: Bordetella IgA, IgG, IgM; Borrelia IgG, IgM; Chlamydia IgA, IgG; COVID IgA, IgG, IgM; CMV IgG, IgM; EBV IgA, IgG, IgM; Helicobacter IgA, IgG; HSV 1+2 IgG, IgM; Mycoplasma IgA, IgG, IgM; Yersinia IgA, IgG

- **What is the difference between ANA and ANA Plus, and why can't I work with ANA Plus in some countries? Does this put me at a disadvantage in terms of evaluation and interpretation?**
  - Difference is in one missing antigen because that is patented in France, Switzerland, Great Britain, Germany, Sweden, Spain, and Italy.
  - The missing antigen is HMGCR – marker of statine induced necrotizing myopathy
  - It does not put you in disadvantage because the kit has 43 more antigens that are perfectly enough to evaluate the sample.

- **Why are there test kits with only 48 tests?**
  - 48 tests have kits for less frequent parameters such as Mycoplasma, Helicobacter, Yersinia, and Bordetella.

CERTIFICATION/STABILITY/STORAGE

- **Do all available parameters have the CE certificate and are IVDR approved?**
  - All available parameters have the CE certificate.
  - CMV and HSV kits are IVDR certified.
  - All tests placed on the market prior to 26 May 2022 have *legacy devices* status. They are in so-called transition period and are still IVDD. They will be in this transition period mostly until 2025-2027, depending on the test type.

- **What is the shelf life of the kits and how are they stored?**
  - They are to be stored in a fridge in 2-8°C.
  - Standardly, it is 12 months.

  - For kits that have been on the market for a longer time, according to a stability study, it is often 18 months.

- **Which EQAs are recommended for the test?**
  - INSTAND (DE), Labquality (FI), CAP (USA)

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