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Instructions for use

HISTO SPOT[®] HLA AB Screen/ID Kits

Test kits for the identification of anti-HLA antibodies on a multiplex immunodiagnostic basis

Electronic instructions for use see www.bag-healthcare.com



REF 728100: HISTO SPOT[®] HLA AB ID Class I (48 tests)

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REF 728101: HISTO SPOT[®] HLA AB ID Class II (48 tests)

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REF 728102: HISTO SPOT[®] HLA AB Screen/ID Class I (480 tests)

REF 728103: HISTO SPOT[®] HLA AB Screen/ID Class II (480 tests)

REF 728198: HISTO SPOT[®] AB Reagent Kit (96 tests)

Contents

1.	PRODUCT DESCRIPTION.....	2
2.	TEST PRINCIPLE.....	2
3.	MATERIAL.....	3
3.1	Reagents provided with the HISTO SPOT [®] HLA AB Screen/ID kits specific for HLA class I and class II	3
3.1.1	HISTO SPOT [®] AB ID Class I kit.....	3
3.1.2	HISTO SPOT [®] AB ID Class II kit.....	3
3.1.3	HISTO SPOT [®] AB Screen / ID Class I kit.....	3
3.1.4	HISTO SPOT [®] AB Screen / ID Class II kit.....	3
3.2	Reagents provided with the HISTO SPOT [®] AB Reagent kit	4
3.3	Reagents and equipment required but not provided.....	4
4.	STORAGE AND STABILITY	4
5.	TEST PROCEDURE.....	4
5.1	Safety conditions and special remarks.....	4
5.2	Sample preparation.....	5
5.3	Automated assay on the MR.SPOT [®] processor.....	5
5.3.1	Reagent preparation	5
5.3.2	Sample / Control Dilution	6
5.3.3	Setup of the MR.SPOT [®] processor	7
5.3.4	Transfer of results to a PC for interpretation	7
5.3.5	Interpretation of results	8
6.	WARNINGS AND PRECAUTIONS	9
7.	SPECIFIC PERFORMANCE CHARACTERISTICS	10
7.1	Evaluation.....	10
7.2	Assay resolution	10
8.	LIMITATIONS OF THE METHOD	10
9.	INTERNAL QUALITY CONTROL.....	10
10.	TROUBLESHOOTING.....	11
11.	TRADEMARKS USED IN THIS DOCUMENT/PRODUCT	11
12.	EXPLANATION OF SYMBOLS USED ON LABELING	12

1. PRODUCT DESCRIPTION

The HISTO SPOT[®] HLA AB Screen/ID system is an in vitro diagnostic test for identification of HLA antibodies on a multiplex immunodiagnostic basis and provides high resolution antibody (Ab) identification results. It consists of the HISTO SPOT[®] HLA AB screening and identification kits, the HISTO SPOT[®] AB Reagent kit, the MR.SPOT[®] processor and the HISTO MATCH HLA AB module interpretation software.

The HISTO SPOT[®] HLA AB screening and identification kits contain testwells with immobilized HLA Class I or Class II antigens for the detection of specific anti-HLA antibodies, a negative control and a positive control. The HISTO SPOT[®] AB Reagent kit contains the reagents required for incubation and detection of bound antibodies and can be used in combination with all HISTO SPOT[®] HLA AB screening and identification kits. The MR.SPOT[®] processor is specifically designed to be used with the HISTO SPOT[®] kits in order to process between 1 and 96 samples, automating the process from incubation, detection through to result interpretation. The HISTO MATCH software is required to interpret the results.

2. TEST PRINCIPLE

The test includes four basic steps:

- sample preparation
- incubation
- detection
- data interpretation

The HISTO SPOT[®] HLA AB Screen/ID test is a miniaturized and multiplexed solid phase enzyme linked immunosorbent assay. Each well of the microtiter plates is printed with various recombinant or native HLA antigens. Each antigen is printed on the bottom of the well to have an independent spot.

After dilution, the sera are transferred in the testwells containing an array of immobilized HLA antigens. In the subsequent incubation time anti-HLA antibodies present in the serum bind to their specific antigens. In a first wash step non-specific antibodies are removed. Following this wash step, a specific peroxidase -labelled antibody directed against human IgG (conjugate) is added in the wells to bind to the present antibody-antigen complexes in the subsequent incubation time. Afterwards wash buffer is added to the wells to remove unbound conjugate. Then a TMB substrate is added to each well. If peroxidase is present a enzyme-substrate reaction takes place which causes a blue colouring of the spots. The dots in the bottom of each test well are photographed by the MR.SPOT[®] processor and the image is transferred into the HISTO MATCH HLA AB module software installed on the PC of the user. The image analysis program of the HISTO MATCH HLA AB module software determines the intensity of each spot in the array and compares it to the intensity of the background. From this data the positive and negative reactions are calculated. The pattern matching program of the HISTO MATCH HLA AB module software identifies the HLA antibodies of the sample against the HLA antigens of the pattern in the array.

3. MATERIAL

3.1 Reagents provided with the HISTO SPOT® HLA AB Screen/ID kits specific for HLA class I and class II

3.1.1 HISTO SPOT® AB ID Class I kit

Testwells Abs Class I	8-well strips , individually packed, each strip containing 8 tests, contains immobilized HLA Class I antigens
------------------------------	--

CONTROL +	Positive control , concentrated solution, to be diluted (pink cap)
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CONTROL -	Negative control , ready to use
------------------	--

With each kit there is a CD containing the batch file that has to be stored within the database of the interpretation software and the instructions for use.

3.1.2 HISTO SPOT® AB ID Class II kit

Testwells Abs Class II	8-well strips , individually packed, each strip containing 8 tests, contains immobilized HLA Class II antigens
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CONTROL +	Positive control , concentrated solution, to be diluted (blue cap)
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CONTROL -	Negative control , ready to use
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With each kit there is a CD containing the batch file that has to be stored within the database of the interpretation software and the instructions for use.

3.1.3 HISTO SPOT® AB Screen / ID Class I kit

Testwells Abs Class I	96-well plates , individually packed, each plate containing 96 tests, contains immobilized HLA Class I antigens
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CONTROL +	Positive control , concentrated solution, to be diluted (pink cap)
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CONTROL -	Negative control , ready to use
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With each kit there is a CD containing the batch file that has to be stored within the database of the interpretation software and the instructions for use.

3.1.4 HISTO SPOT® AB Screen / ID Class II kit

Testwells Abs Class II	96-well plates , individually packed, each plate containing 96 tests, contains immobilized HLA Class II antigens
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CONTROL +	Positive control , concentrated solution, to be diluted (blue cap)
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CONTROL -	Negative control , ready to use
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With each kit there is a CD containing the batch file that has to be stored within the database of the interpretation software and the instructions for use.

3.2 Reagents provided with the HISTO SPOT® AB Reagent kit

The reagents contained in one kit are sufficient for 96 tests. Each reagent set contains:

SAMBUF	Sample Buffer , ready to use, contains 0.05% Proclin® 300
WASHBUF	Wash Buffer , ready to use, contains 0.05% Proclin® 300
CONJ AB	Conjugate , Anti-human IgG conjugate with horseradish peroxidase, contains 0.05% Proclin® 300
CONJBUF	Conjugate diluent Buffer , ready to use, contains 0.05% Proclin® 300
SUBS AB	Substrate , ready to use, (3,3',5,5'-Tetramethylbenzidine)

3.3 Reagents and equipment required but not provided

- MR.SPOT® processor **REF** 726100
- HISTO MATCH software, **REF** 726102
- Pipette tips for the MR.SPOT® processor, 1000 µl **REF** 726099 and 200 µl **REF** 726097
- Sample plate: Skirted PCR plates with lids or adhesive film (HISTO SPOT® PCR Frameplates, **REF** 726220, HISTO SPOT® PCR Caps, **REF** 726090, HISTO SPOT® PCR Foils, **REF** 726089)
- Deionized water
- Variable pipettes (range 0.5 – 1000 µl) and disposable tips

4. STORAGE AND STABILITY

All reagents and kit components have to be stored at 2...8°C.

The expiry date is indicated on the label of each reagent and is valid for the originally sealed reagents. The expiry date indicated on the outer box label refers to the reagent with the shortest stability contained in the kit.

Assay performance is dependent on reagents stability, those can not be used after their expiration date.

Testwells stored in open foil pouches **can be used up to 7 days after opening**.

The conjugate dilution must always be **prepared afresh** for each test run.

5. TEST PROCEDURE

5.1 Safety conditions and special remarks

The HISTO SPOT® HLA AB test should be performed by well trained and authorized laboratory technicians. The HISTO SPOT® HLA AB kits can only be used in combination with the HISTO SPOT® AB Reagent kit, the MR.SPOT® processor and the HISTO MATCH HLA AB module software.

The results from these tests must not be used as the sole determinant for making clinical decisions.

Special safety conditions must be noted in order to avoid contamination and thus false reactions:

- ◆ Wear gloves during work (powder-free, if possible).
- ◆ Use new tips with each pipeting step (with integrated filter).
- ◆ Use devices and other materials only at the respective places and do not exchange them.

All the reagents and troughs used for the assay must be those indicated for the HISTO SPOT[®] HLA AB test. The reagents and troughs indicated for SSO DNA testing must be excluded from the processor when a HISTO SPOT[®] HLA AB test is running.

5.2 Sample preparation

Serum samples must be used. Samples collected with anti-coagulant can not be used. EDTA treatment of the serum is consistent with the assay. Samples heat inactivated and samples with microbial contamination may give nonspecific reactions and should not be used. Lipaemic or hemolyzed samples should be avoided.

Serum must be stored at -20°C prior testing. Avoid repeated freezing and thawing of the samples.

Take samples out of the freezer and allow them to warm to room temperature. Samples must be clarified by centrifugation (from 1 to 5 min, from 2500 to 10 000 g) prior to testing.

If there is a fatty layer on the serum after centrifugation this has to be removed or the clearer part of the sample has to be withdrawn and used for the testing.

Vortex the clarified sample before dilution.

5.3 Automated assay on the MR.SPOT[®] processor

5.3.1 Reagent preparation

Take HISTO SPOT[®] AB reagents and HISTO SPOT[®] HLA AB testwells out of the fridge and allow them to warm to room temperature.

Homogenize the reagents **SAMBUF** and **CONJBUF** by shaking before use.

Vortex the **CONTROL** - before use.

Salt crystals may be observed in the Wash Buffer (**WASHBUF**). **Before using warm the Wash Buffer 15 minutes at 30°C to dissolve.** Warm the whole content of the bottle, not an aliquot. Check by eyes if there are no residual crystals remaining, warm it again if it is the case.

The conjugate has to be diluted **1:100** in Conjugate Buffer prior to testing.

The conjugate dilution must always be prepared afresh for each test run.

The conjugate has to be vortexed and spun down each time before the dilution step!

The required volumes of the reagents will vary depending on the number of strips to be tested. The MR.SPOT[®] processor displays the required volumes for the chosen number of strips. Fill the required volumes of the reagents into the **corresponding labelled reservoirs**.

Reagents used for a run **can not be reused** for a second run. After each run, all the reagents should be discarded.

5.3.2 Sample / Control Dilution

It is possible to incubate simultaneously **Testwells Abs Class I** and **Testwells Abs Class II** in the same RUN but with caution on the volumes and the arrangement of the different sera in the plate. This step must begin when all the reagents are prepared and MR.SPOT[®] is ready to use.

- **HLA AB ID Class I:** add **5 µl of the serum** and **95 µl of SAMBUF** in the sample plate.
Mix at least 5 times or vortex and centrifuge the sample plate after dilution.
and/or
- **HLA AB ID Class II:** add **2 µl of the serum** and **98 µl of SAMBUF** in the sample plate.
Mix at least 5 times or vortex and centrifuge the sample plate after dilution.

For each run and each assay (Class I and Class II) **CONTROL +** and **CONTROL -** must be added.

- **CONTROL +** has to be diluted at **1:50** before use:
add **1 µl of CONTROL +** in **50 µl of SAMBUF**, vortex
- **CONTROL - NC01** and diluted **CONTROL + PC01 (Class I)** are used as a sample,
add **5 µl of CONTROL -** resp. **CONTROL +** and **95 µl of SAMBUF** in the sample plate.
Mix at least 5 times or vortex and centrifuge the sample plate after dilution.
- **CONTROL - NC02** and diluted **CONTROL + PC02 (Class II)**, are used as a sample,
add **2 µl of CONTROL -** resp. **CONTROL +** and **98 µl of SAMBUF** in the sample plate.
Mix at least 5 times or vortex and centrifuge the sample plate after dilution.

The serum sample dilutions given above (1:20 for class I and 1:50 for class II) haven been validated and give optimal results with most of the tested samples. If the sera show very weak or very strong reactions other dilutions might be better. If the results are unclear it is recommended to titrate the sera.

Place the sample plate with the diluted sera and controls in the sample plate holder in the MR.SPOT[®] processor. Be careful to do this with the correct orientation of the sample plate.

Place the testwells into the reaction plate holder of the MR.SPOT[®] processor.

Note the correct arrangement of the testwells according to the worklist layout.
Please make sure that there is no dirt or plastic particles in the reaction plate holder.

The testwells can be separated in single wells according to figure 1, if less than eight tests should be run. If you are using separated wells be careful that they are sitting properly in the reaction plate holder and are not twisted against each other. **Clear dummy wells have to be added to reach test numbers of multiples of four.**

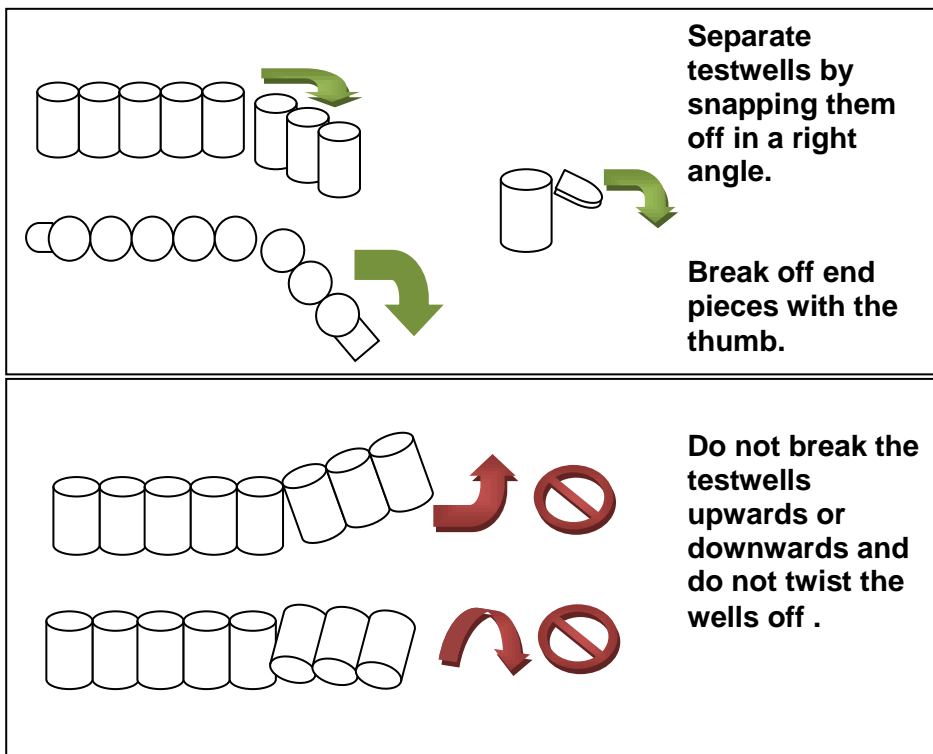


Fig. 1: Separating single testwells

5.3.3 Setup of the MR.SPOT[®] processor

Switch on the MR.SPOT[®] processor. The start up screen will appear. Follow the process as indicated on the screen. Details are described in the User Manual for the MR.SPOT[®] processor. The temperature in the processor must not exceed 30°C.

5.3.4 Transfer of results to a PC for interpretation

Transfer the data to the HISTO MATCH HLA AB module software via network or USB stick as described in the manual for the HISTO MATCH software.

5.3.5 Interpretation of results

Open the HISTO MATCH HLA AB module software (if this is not already installed, it can be installed from the CD delivered with the MR.SPOT[®] processor) and interpret the data as described in the manual for the HISTO MATCH software.

The images should look like the example shown in figure 2. Figure 3 gives a schematic illustration of the result and the functions of the different probes.

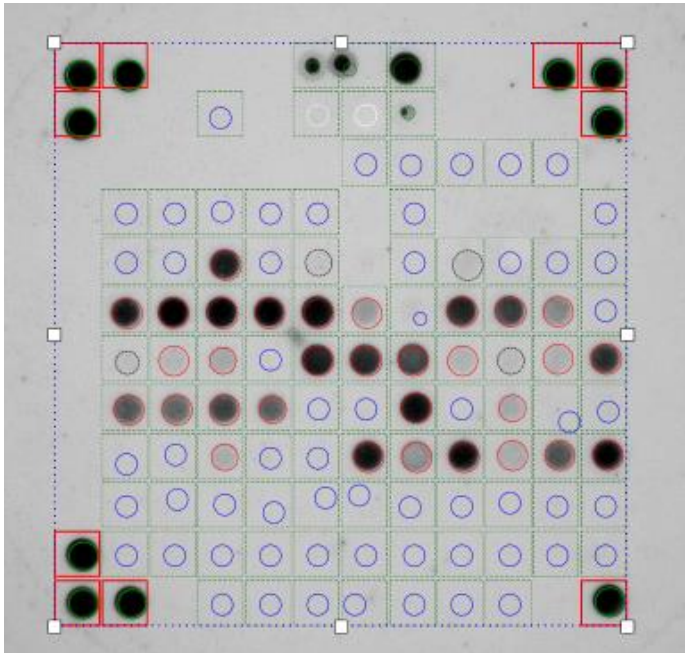


Figure 2: Example for a sample containing anti-HLA Class I antibodies.

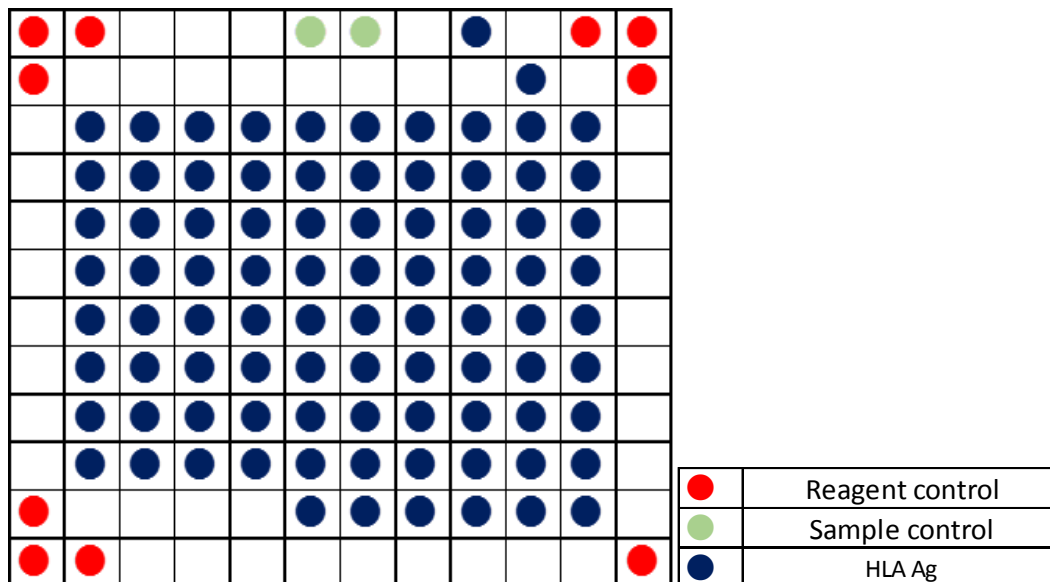


Figure 3: Schematic representation of the HISTO SPOT[®] HLA AB ID Class I

The interpretation of the spots performed by the software must be checked visually by the operator.

The automatic interpretation is meant as a tool to aid the user and must be validated by checking the image and the plausibility before assigning any result.

In the case of non-valid results due to e.g. dust or smear the operator can move the spot area in the grid or disable the spot (please see the instructions for use for the HISTO MATCH HLA AB module for details).

If a patient serum reacts positive with more than 80% of the antigens on the testwell on the microarray, the results are considered uninterpretable as these positivites can be considered as non-specific. A new test must be performed at a higher dilution (e.g. **1:100 for Class I** and **1:200 for Class II**) to avoid non-specific interaction and put in evidence the specific interaction.

6. WARNINGS AND PRECAUTIONS

HISTO SPOT[®] is designed for in vitro diagnostic use and should be used by properly trained, qualified staff. All work should be performed using Good Laboratory Practices.

Biological material used, e.g. sera, should be handled as potentially infectious. When handling biological material appropriate safety precautions are recommended (do not pipet by mouth; wear disposable gloves while handling biological material and performing the test; disinfect hands when finished the test).

Biological material should be inactivated before disposal (e.g. in an autoclave). Disposables should be autoclaved or incinerated after use.

Spillage of potentially infectious materials should be removed immediately with absorbent paper tissue and the contaminated areas swabbed with a suitable standard disinfectant or 70% alcohol.

Material used to clean spills, including gloves, should be inactivated before disposal (e.g. in an autoclave).

SAMBUF, **WASHBUF** and **CONJBUF** contain ProClin[®]300 in a very low concentration of 0.05% as preservative, nevertheless avoid contact with the skin and mucous membranes.

Do not expose **SUBS AB** to metals, oxidising agents. **SUBS AB** may possess skin sensitizing and shlightly polluting properties.

All work with reagents should be handled with the appropriate precautions. Wear eye protection, laboratory coats and disposable gloves when handling the reagents. Avoid contact of these materials with the skin, eyes or mucous membranes. If contact does occur, immediately wash with large amounts of water. Burns can occur if left untreated.

If spills of reagents occur, dilute with water before wiping dry.

Disposal of all samples, unused reagents and waste should be in accordance with country, federal, state and local regulations.

Avoid microbial contamination of reagents when removing aliquots from reagent bottles. The use of sterile disposable pipettes and pipette tips is recommended. Do not use reagents with evidence of turbidity or microbial contamination.

To avoid bacterial growth in the tubing, the MR.SPOT[®] processor must be disinfected monthly with MR.SPOT[®] disinfectant following the procedure indicated on the manual.

Material Safety Data Sheets (MSDS) are available to download at www.bag-healthcare.com .

7. SPECIFIC PERFORMANCE CHARACTERISTICS

7.1 Evaluation

Evaluation studies have been performed for the HISTO SPOT[®] HLA AB kits using samples characterized with CDC or other SA solid phase assays. Each batch is released after a quality control using internal referenced samples.

7.2 Assay resolution

The HISTO SPOT[®] HLA AB tests are designed to detect and determine HLA antibodies on the two fields level of resolution.

8. LIMITATIONS OF THE METHOD

Extreme care should be taken to prevent bacterial contamination of the kit reagents and other laboratory materials and equipment.

Erroneous results can occur if inadequate reagents, temperature, incubation period or washing are used. Therefore, the HISTO SPOT[®] HLA AB tests should only be used in combination with the MR.SPOT[®] processor to ensure correct temperatures and incubation times.

All instruments (e.g. pipettes, MR.SPOT[®] processor) must be calibrated according to the manufacturers instructions.

Samples from patients with autoimmune disorders or receiving immunoglobulin therapy have not to be tested. Such samples may give false positive or false negative results.

If a patient serum reacts positive with more than 80% of the antigens on the testwell, a retest must be performed at a higher dilution, to avoid false positive interaction.

Some very low titer antibodies to HLA antigens may not be detected and may give false negative results.

The pretreatment of the sample that are not listed in the preparation section must be validated by the laboratory prior testing.

9. INTERNAL QUALITY CONTROL

Internal positive controls are contained in each testwells to ensure successful incubation and reagent dispensing.

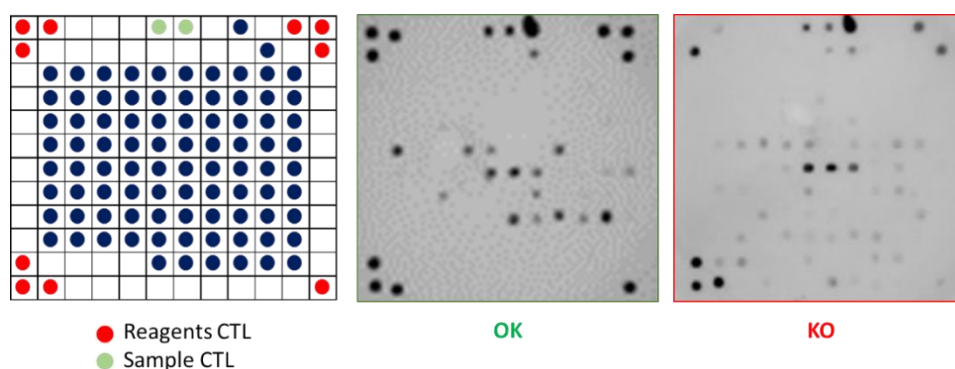


Figure 4: Control checking before the validation of the assay (CTL = Control)

The operator must check on the picture that all the CTL spots are positive before assigning a result. In case of low or no spots for IgG or anti-IgG CTL, the result can not be interpreted.




10. TROUBLESHOOTING

Symptom	Possible problem(s)	Potential Solution(s)
Instrument malfunction	Numerous	Refer to MR.SPOT [®] manual
Error message at data transfer	Failure in data transfer	Manually transfer data using USB drive
No result	Failure to grid image	Perform manual gridding
No spots in the well	Failure to add a reagent	Repeat whole assay
No result / inconclusive result due to weak signals	Mistake in conjugate dilution Instrument malfunction	Repeat assay. Check instrument

11. TRADEMARKS USED IN THIS DOCUMENT/PRODUCT

Proclin[®] is a trademark of Rohm and Haas company.

12. EXPLANATION OF SYMBOLS USED ON LABELING

	Storage temperature
	Use by
	Consult instructions for use
CLASS I	HLA Class I
CLASS II	HLA Class II
CONJ AB	Anti-human IgG conjugate (horseradish peroxydase)
CONJBUF	Conjugate diluent buffer
CONTROL +	Positive control
CONTROL -	Negative control
HLA ANTIBODY ID	Intended use: HLA antibody identification
IVD	For in vitro diagnostic use
LOT	Batch code
REF	Catalogue number
SAMBUF	Sample buffer
SUBS AB	Substrate
Testwells Abs Class I	Testwells with printed HLA Class I Antigens
Testwells Abs Class II	Testwells with printed HLA Class II Antigens
WASHBUF	Wash buffer
HISTO SPOT® HLA AB Information CD	CD, contains the Instructions for use and with the HISTO SPOT® HLA AB Screen/ID kits additionally the Batch File

Instructions for use in other languages see <http://www.bag-healthcare.com>
<http://service.bag-healthcare.com> or phone +49 (0)6404-925-125