MegaFLUO®

Possible Sources of Trouble

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Weak or no fluorescence of the Positive Control

1. The Controls available at **MEGACOR Diagnostik GmbH** are aliquoted in ready to use dilutions already. The dilution was optimised for the respective test. To obtain a strong reaction in every case, the Positive Controls must not be diluted further.
2. The most important role of the Positive Control is the recognition of the respective fluorescence pattern, the intensity is only secondary.
3. The microscope must be equipped with a FITC filter system (maximal excitation wave lenght 490 nm, average emission wave lenght 530 nm) and a 400 fold magnification.
4. Reason for a weak reaction of the Positive Control can also be the wrong handling of the Conjugate. This should always be stored in the dark at 2–8 °C and should only be obtained from the lightsaving package shortly before use. The slide should be incubated in the wet chamber in the dark, because daylight or artificial light can damage the fluorescent marker of the Conjugate.
5. The test-kit component (apart from conjugate!) should have room temperature at the time of application.
6. For washing procedure we recommend a freshly made PBS dilution without magnesium, calcium or Tween. The procedure should be performed as described in the instructions for use. The slides should be rinsed in distilled water after each washing step, because traces of salts can cause background.
7. It is quite possible to wash the slide too thoroughly. Therefore, we recommend using a washing bottle and not to focus the stream directly onto the antigen wells.
8. The use of the Mounting Medium is necessary to stabilise the activity of the Conjugate for a longer time. If you use an amount too small, air bubbles can arise on the slides that cannot be pushed away any more. This complicates the evaluation (different levels and intensities of the fluorescence). Without Mounting Medium, the FITC Conjugate disintegrates under exposure onto UV light.

Sample cannot be evaluated

1. Some sera have a strong green non-specific background staining (“greening effect”). These sera can fluoresce and show their pattern of fluorescence only in very high dilutions. Therefore, we recommend to forego the point titration and try to test various dilutions around the cut off.

Antigens or cells smear or are already missing on the slide before testing

1. The slides must adjust to room temperature before opening the aluminium pouches. In opened pouches with cold or frozen slides, condensate is forming rapidly. Then, an antigen suspension can be forming running from the slides. These slides are useless. Lay the slides separately (not piled) in their pouches for 15 to 20 minutes on the laboratory table.
2. The vacuumed pouches are tested routinely for cracks before transport. If you got slides with non-functioning vacuum pouches, please inform your distributor.

Antigens or cells are washed from the slide during the test

1. It is quite possible to wash the slide too thoroughly. Therefore, we recommend using a washing bottle and not to focus the stream directly onto the antigen wells.
2. If only some sera tend to loosen the substrate from the antigen well, then a bacterial protease can be the cause. It is present when there is bacterial growth in the serum.
3. If the substrate loss is patched or in stripes, the substrate was affected or scratched during application of serum or conjugate. Sera and conjugates should be applicated from the margin of the antigen well.

Negative Control is positive

1. For the washing step, we recommend using a washing bottle and not to focus the stream directly onto the antigen wells. Diluted sera running over a neighboring well can influence the fluorescence reaction of this well, especially with highly positive sera placed next to negative sera (e. g. Negative Control).
2. The same problem occurs when the different serum samples on the wells are mixed by jerky movement of slides or incubation chamber.

Fluorescence reaction is stronger at the margin than in the middle

1. This phenomenon depends on cell distribution in each antigen well and on the so-called droplet effect. Because the margin of the well normally shows less antigen, the reaction seems to rise due to enhancement of the antibody-antigen relation. This effect also is enhanced by the kinetic energy coupled with the surface tension at the margin of the serum drop. We recommend to always determine the fluorescence intensity in the middle of the antigen well.

Background

1. For washing procedure we recommend a freshly made PBS dilution without magnesium, calcium or Tween. The procedure should be performed as described in the instructions for use. The slides should be rinsed in distilled water after each washing step, because traces of salts can cause background.
2. The incubation times have to be complied precisely. Longer incubation times may cause background.
3. The wells must not dry during the whole test procedure.
