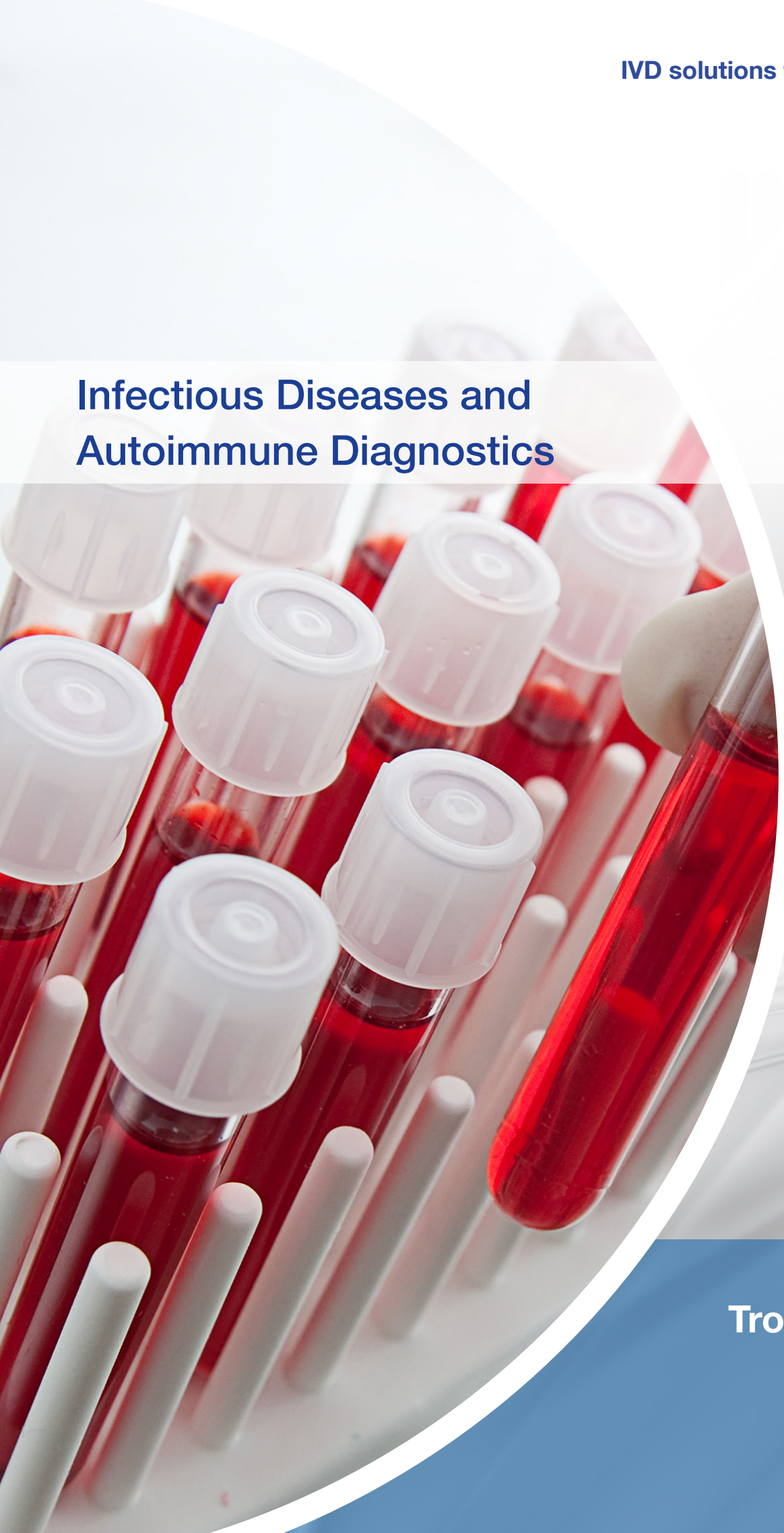
IVD solutions through partnership

Mast Group

Infectious Diseases and Autoimmune Diagnostics



Troubleshooting ELISA

No colour reaction

Conjugate

Reagent based

- Conjugate contaminated
  - With substrate
  - Direct contact with sodium azide
  - Cross contamination with sodium azide stabilised reagents e.g. using the same tip for control sera and conjugate

Consequence: No enzyme activity

- Cleavage of the binding of enzyme or antibodies
  - Caused by chemical reaction e.g. pH shift
  - Due to concentrated wash solution

The conjugate is diluted in red-coloured diluting buffer and adjusted to the required working concentration by the manufacturer. The red colour may vary slightly form lot to lot due to slight pH shifts to lower pH.

Handling based

- Use of incorrect conjugate e.g. from another kit with different concentration
- Use of incorrect conjugate (IgG, IgM or IgA)

Substrate, TMB

Reagent based

Blue greenish substrate due to probably contamination with HCl or H_2SO_4.

Consequence: No colour reaction because the substrate can not be modified enzymatically.
Ready to use TMB should be a clear and nearly colourless liquid. In case the substrate shows a greenish colour, it was probably stored at too low temperatures which results in lower activity. In case of doubt it should be thrown out because the colour change could also be due to contamination resulting in an activity loss.

If the substrate is stored at higher temperature, you will notice a colour change to yellow. Here the substrate will be active.

Handling based

- Washing step after substrate reaction
- Pipetting of a wrong reagent e.g. stop solution instead of substrate

Washing buffer

The concentrated washing buffer must be diluted (MASTAZYME ELISA 1:10). Contaminated or not properly purified water may block the enzymatic activity. Even water purified by ion exchange chromatography may cause troubles because some aliphatic or aromatic components are not eliminated.
Recommendation: Double distilled water or aqua iniectabilia

Handling based

- Wrong dilution of the washing buffer e.g. concentrated wash buffer can remove bound antibodies (Ab) or antigens (Ag)
- Too intense washing in particular “bottom wash” of some instruments may remove bound Ab or Ag
- In case of automated washing, the dispenser may scrape off the antigen of the plate bottom

Sample

Do not use lipaemic, icteric or haemolytic samples. These samples may interfere in the assay and may give false result. Ask for a new sample.

Equivocal colour reaction

Weak colour reaction

Reagent based

- Contaminated reagents e.g. with sodium azide, but still show some active reactivity
- Damaged package of the strips leads to partial or complete denaturation of the antigen due to moisture
- Temperature of the washing buffer is above 24 °C which results in a higher washing efficiency that may remove bound Ag or Ab

Handling based

- Strips are taken out of the fridge, immediately opened and used without reaching room temperature , e.g. this delays the binding of Ab from the sample
- Strips are taken back into package but the package is not properly resealed. Enclosed moisture may result in denaturation of the coated antigen
- Use of reagents not reached room temperature delays binding of Ab or conjugate
- Application of a non-original kit conjugate or substrate
- Extended soaking of washing buffer in the wells e.g. running the assay on wash systems may remove bound Ag or Ab
- Tap out residual washing buffer to avoid interference of washing buffer with conjugate or diluting the substrate
- Wrong height adjustment of the manifold in an automated washer may wash of bound Ag or Ab
- Incorrect incubation times e.g. for sample incubation too short incubation time lowers the binding of Ab to Ag, for conjugate it reduces the binding and for substrate it slows down the substrate turnover

Too strong colour reaction

Handling based

- Application of a conjugate not supplied with the kit
- Washing intensity was not strong enough, e.g. unspecific sticky sample Ab remain attached to the MTP surface
- Washing after conjugate incubation was not intense enough e.g. remaining conjugate cause colour development
- Residual liquid after washing after conjugate addition may react with substrate
- Extended incubation time particularly the substrate incubation results in an elevated colour development
- Incubation temperature exceeds 24 °C (e.g. direct sun light) e.g. increases number of bound Ab, conjugate and speeding up substrate turnover

Sample based

- Samples, containing anti-BSA-antibodies may cause strong positive reactions
- Some coated antigens may still show enzymatic activity which cause colour change of the substrate despite lacking specific antibodies e.g. myeloperoxidase (MPO) or thyroid peroxidase (TPO)
- Specific samples may show higher background due to non-specific enzyme activity e.g. MPO

Wrong positive or negative samples

Reagent based

- Contaminated sample diluent
- Samples may be haemolytic, icteric or lipemic
- Calibrators or cut-off controls are not stable and show decreasing optical density e.g. due to storage at room temperature, transport at elevated temperatures

Elevated OD blank

Handling based
- Wrong sample diluent
- Wrong or no sample dilution
- Wrong washing
- Wrong filter selection in the photometer

Sample based
- Antibody degradation due to elevated temperatures during transport
- Antibody degradation due to contamination

Reagent based
- Contaminated reagents
- Inappropriate water selected (double distilled water or aqua iniectabilia for HPLC is recommended)

Handling based
- Wrong washing
- Residue on the the bottom of ELISA plate

Precision problems

Strong variation of results (Intra-/ Interassay)

Reagent based
- Damaged pouch of the ELISA plate
- Contaminated reagents

Handling based
- Reagents particularly the ELISA plate and samples are not equilibrated at room temperature before starting the assay esp. edge effect
- Samples are not thoroughly mixed after dilution
- Little air bubbles in the wells
- Pipette tips do not fit properly – slightly wobbling
- Contaminated pipette tips
- Blocking of some washer tips
- ELISA plate dries in during incubation or after washing steps
- Strips are not fixed properly in the strip frame resulting in irregular washing

Comments:
In case of intra and inter-assay variation it has to be clarified if OD (450 nm) or quantitative results are affected. Results of OD are directly influenced by e.g. temperature; incubation time or humidity that affects calibrators, controls and samples in equal measure the obtained results is generally corrected. High variation for inter-assay variation of the quantitative results is often caused by crucial mistakes in the reagent system or handling.

V. 2019-02-11

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