CLAIMS

1. Assay method for determining the potency of anti-MIF antibodies, wherein a cell migration is performed, wherein the assay method comprises the following steps:
   - providing cells which are capable of migration in a first part of a device, wherein the cells comprise MIF,
   - adding the anti-(ox)MIF antibody to be determined, to a second part of the device, which is configured to be in a connection with the first part of the device which allows cell migration and diffusion, and
   - calculating inhibition of migration.
2. Assay method according to claim 1, wherein the anti-MIF antibodies are anti-oxMIF antibodies, preferably wherein the antibodies are selected from the group consisting of RAB0, RAB4, RAB9, RAM0, RAM4 and/or RAM9, very preferred RAM9.
3. Assay method according to claim 1 or 2, wherein the device comprises an upper and a lower chamber, which are connected via a separating membrane with pores and in that the cells are added to the upper chamber and in that the antibodies are added to the lower chamber, preferably wherein the device is a two chamber cell migration assay (modified Boyden chamber assay), more preferably a Transwell cell migration assay device.
4. Assay method according to any one of the preceding claims, wherein the cells are cells which express (ox)MIF, preferably on their cell surface, preferably wherein the cells are cells from disease samples, more preferred wherein the cells are monocytic cells, preferably human monocytic cells, more preferred human immortalized monocytic cells, most preferred U937 cells, or THP-1 human monocytic cells or in an alternative embodiment rat NR 8383 monocytic cells.
5. Assay method according to any one of the preceding claims, wherein the cells are provided as a cell suspension in a suitable migration medium to allow migration.
6. Assay method according to claim 5, wherein the migration medium does not comprise proteins.
7. Assay method according to any one of the preceding claims, wherein the antibody is added in a non toxic biological buffer with a moderately weak acid and its conjugate base, preferably a glycine buffer, an N-substituted taurine buffer or a phosphate buffered saline (PBS) buffer, to the second part, e.g. lower chamber, of e.g. the Transwell® chamber.
8. Assay method according to any one of the preceding claims, wherein the antibody is added to have a final concentration in the assay of 0.01 – 100 nM, preferably 0.02 to 50 nM, preferably 0.04-30 nM, preferably as a dilution series
9. Assay method according to any one of the preceding claims, wherein the assay, e.g. the Transwell® chamber, is incubated after addition of the cells and the antibody for 6-20 h, preferably 8-12 h, preferably at approximately 37°C, wherein the cells are U937 cells, and wherein the membrane has a pore size of approximately 5 µm.
10. Assay method according to any one of the preceding claims, wherein the migrated cells are counted, preferably after the above incubation step, and preferably in the lower chamber, whereupon

information about the potency of the tested antibody can be obtained, preferably by calculating the half-maximal inhibiting antibody concentration (IC_{50}-value).

11. Assay method according to any one of the preceding claims, wherein the cells undergo from 10 to 48 h, preferably 8-16, preferably 10-12 h serum starvation before they are used in the assay.

12. Assay method according to any one of the preceding claims, wherein the cells are derived from a working bank.

13. Assay method according to any one of the preceding claims, wherein no (ox)MIF is added to the device.

14. Assay kit for determining the potency of anti-(ox)MIF-antibodies, comprising all reagents necessary to carry out the assay method of any one or more of the preceding claims, preferably
    - cells which express MIF
    - antibody dilution buffer (e.g. glycine buffer)
    - migration medium, and/or
    - Two chamber cell migration system.

15. Pharmaceutical or diagnostic composition, comprising anti-(ox)MIF antibodies, wherein the anti-(ox)MIF antibodies are characterized in that they have undergone a potency determination, as defined in any one of the precedings claims.

AMENDED CLAIMS

received by the International Bureau on 01 April 2014 (01.04.2014)

1. Assay method for determining the potency of anti-MIF antibodies, wherein a cell migration is performed, wherein the assay method comprises the following steps:
   - providing cells which are capable of migration in a first part of a device, wherein the cells comprise MIF,
   - adding the anti-(ox)MIF antibody to be determined, to a second part of the device, which is configured to be in a connection with the first part of the device which allows cell migration and diffusion, and
   - calculating inhibition of migration, wherein the device comprises an upper and a lower chamber, which are connected via a separating membrane with pores and in that the cells are added to the upper chamber and in that the antibodies are added to the lower chamber and wherein no (ox)MIF is added to the device.
2. Assay method according to claim 1, wherein the anti-MIF antibodies are anti-oxMIF antibodies, preferably wherein the antibodies are selected from the group consisting of RAB0, RAB4, RAB9, RAM0, RAM4 and/or RAM9, very preferred RAM9, wherein RAB0 is defined by co-expression of plasmids DSM 25115 and DSM 25114, RAB4 is defined by co-expression of plasmids DSM 25110 and DSM 25112, RAB9 is defined by co-expression of plasmids DSM 25111 and DSM 25113, RAM0 is defined by co-expression of plasmids DSM 25863 and DSM 25864, RAM4 is defined by co-expression of plasmids DSM 25861 and DSM 25862, and RAM9 is defined by co-expression of plasmids DSM 25859 and DSM 25860.
3. Assay method according to claim 1 or 2, wherein the device is a two chamber cell migration assay (modified Boyden chamber assay), more preferably a Transwell cell migration assay device.
4. Assay method according to any one of the preceding claims, wherein the cells are cells which express (ox)MIF, preferably on their cell surface, preferably wherein the cells are cells from disease samples, more preferred wherein the cells are monocytic cells, preferably human monocytic cells, more preferred human immortalized monocytic cells, most preferred U937 cells, or THP-1 human monocytic cells or in an alternative embodiment rat NR 8383 monocytic cells.
5. Assay method according to any one of the preceding claims, wherein the cells are provided as a cell suspension in a suitable migration medium to allow migration.
6. Assay method according to claim 5, wherein the migration medium does not comprise proteins.
7. Assay method according to any one of the preceding claims, wherein the antibody is added in a non toxic biological buffer with a moderately weak acid and its conjugate base, preferably a glycine buffer, an N-substituted taurine buffer or a phosphate buffered saline (PBS) buffer, to the second part, e.g. lower chamber, of e.g. the Transwell® chamber.
8. Assay method according to any one of the preceding claims, wherein the antibody is added to have a final concentration in the assay of 0.01 – 100 nM, preferably 0.02 to 50 nM, preferably 0.04-30 nM, preferably as a dilution series

9. Assay method according to any one of the preceding claims, wherein the assay, e.g. the Transwell® chamber, is incubated after addition of the cells and the antibody for 6-20 h, preferably 8-12 h, preferably at approximately 37°C, wherein the cells are U937 cells, and wherein the membrane has a pore size of approximately 5 µm.

10. Assay method according to any one of the preceding claims, wherein the migrated cells are counted, preferably after the above incubation step, and preferably in the lower chamber, whereupon information about the potency of the tested antibody can be obtained, preferably by calculating the half-maximal inhibiting antibody concentration (IC_{50}-value).

11. Assay method according to any one of the preceding claims, wherein the cells undergo from 10 to 48 h, preferably 8-16, preferably 10-12 h serum starvation before they are used in the assay.

12. Assay method according to any one of the preceding claims, wherein the cells are derived from a working bank.

13. Assay kit for determining the potency of anti-(ox)MIF-antibodies, comprising all reagents necessary to carry out the assay method of any one or more of the preceding claims, preferably
    - cells which express MIF
    - antibody dilution buffer (e.g. glycine buffer)
    - migration medium, and/or
    - Two chamber cell migration system
    - wherein no (ox)MIF is added to the device.

CLAIMS

1. Assay method for determining the potency of anti-MIF antibodies, wherein a cell migration is performed, wherein the assay method comprises the following steps:
   - providing cells which are capable of migration in a first part of a device, wherein the cells comprise MIF,
   - adding the anti-(ox)MIF antibody to be determined, to a second part of the device, which is configured to be in a connection with the first part of the device which allows cell migration and diffusion, and
   - calculating inhibition of migration.
2. Assay method according to claim 1, wherein the anti-MIF antibodies are anti-oxMIF antibodies, preferably wherein the antibodies are selected from the group consisting of RAB0, RAB4, RAB9, RAM0, RAM4 and/or RAM9, very preferred RAM9.
3. Assay method according to claim 1 or 2, wherein the device comprises an upper and a lower chamber, which are connected via a separating membrane with pores and in that the cells are added to the upper chamber and in that the antibodies are added to the lower chamber, preferably wherein the device is a two chamber cell migration assay (modified Boyden chamber assay), more preferably a Transwell cell migration assay device.
4. Assay method according to any one of the preceding claims, wherein the cells are cells which express (ox)MIF, preferably on their cell surface, preferably wherein the cells are cells from disease samples, more preferred wherein the cells are monocytic cells, preferably human monocytic cells, more preferred human immortalized monocytic cells, most preferred U937 cells, or THP-1 human monocytic cells or in an alternative embodiment rat NR 8383 monocytic cells.
5. Assay method according to any one of the preceding claims, wherein the cells are provided as a cell suspension in a suitable migration medium to allow migration.
6. Assay method according to claim 5, wherein the migration medium does not comprise proteins.
7. Assay method according to any one of the preceding claims, wherein the antibody is added in a non toxic biological buffer with a moderately weak acid and its conjugate base, preferably a glycine buffer, an N-substituted taurine buffer or a phosphate buffered saline (PBS) buffer, to the second part, e.g. lower chamber, of e.g. the Transwell® chamber.
8. Assay method according to any one of the preceding claims, wherein the antibody is added to have a final concentration in the assay of 0.01 – 100 nM, preferably 0.02 to 50 nM, preferably 0.04-30 nM, preferably as a dilution series
9. Assay method according to any one of the preceding claims, wherein the assay, e.g. the Transwell® chamber, is incubated after addition of the cells and the antibody for 6-20 h, preferably 8-12 h, preferably at approximately 37°C, wherein the cells are U937 cells, and wherein the membrane has a pore size of approximately 5 µm.
10. Assay method according to any one of the preceding claims, wherein the migrated cells are counted, preferably after the above incubation step, and preferably in the lower chamber, whereupon

information about the potency of the tested antibody can be obtained, preferably by calculating the half-maximal inhibiting antibody concentration (IC_{50}-value).

11. Assay method according to any one of the preceding claims, wherein the cells undergo from 10 to 48 h, preferably 8-16, preferably 10-12 h serum starvation before they are used in the assay.

12. Assay method according to any one of the preceding claims, wherein the cells are derived from a working bank.

13. Assay method according to any one of the preceding claims, wherein no (ox)MIF is added to the device.

14. Assay kit for determining the potency of anti-(ox)MIF-antibodies, comprising all reagents necessary to carry out the assay method of any one or more of the preceding claims, preferably
    - cells which express MIF
    - antibody dilution buffer (e.g. glycine buffer)
    - migration medium, and/or
    - Two chamber cell migration system.

15. Pharmaceutical or diagnostic composition, comprising anti-(ox)MIF antibodies, wherein the anti-(ox)MIF antibodies are characterized in that they have undergone a potency determination, as defined in any one of the precedings claims.

STATEMENT UNDER ARTICLE 19 (1)

In the following, Applicants provide a statement under Article 19 PCT:

I. Claim 15 - Product by Process

It was objected against this claim: this claim has been deleted and this objection should thus be moot.

II. Claim 14 – Kit Claim; now Claim 13

The kit claim was likewise considered to be not allowable because of an alleged lack of novelty over document **D1**. **D1** is considered to show in Example 16 a transfer assay with all components which would allegedly also be needed according to Claim 14.

In Example 16 of **D1**, anti-MIF antibodies Bax94 and Bax69 were tested in a TransWell invasion assay wherein the human prostate cancer cell line PC-3 was used. The PC-3 were seeded in 24 well-Transwell™ dishes with a pore size of 8 µm, which were coated with poly-D-lysine on the bottom face of the polycarbonate membrane and with a growth-factor depleted matrigel on the Transwell™ insert surface. Cells were allowed to attach for 4 hours in the presence of 10% FCS. Thereafter, the medium was changed to serum-free medium and cells were starved overnight whereupon compounds (10 nM MIF, 500nM antibodies) were added to the lower chamber. Migration through the porous membrane was allowed for 24 hours. The attached migrated cells of the lower face of the membrane were stained.

The present assay however, does not envisage or need the addition of exogenous MIF. This is now also reflected in the language of the kit claim whereby the novelty objection should be overcome.

D2 does not describe the kit or the specific use of the present application and should thus not be pertinent for novelty of this claim.

III. Method Claims 1-12

D1 was considered to be pertinent for novelty or inventive step of the method claims. However, only with the present invention was it possible to provide an assay where no exogenous MIF needed to be added. Apart from this feature, the present claims also differ from the disclosure of D1 in that the cells are added to the upper chamber and the antibodies are added to the lower chamber.

There is no disclosure whatsoever anywhere in D1 or any of the further documents which would point a person skilled in the art to the possibility to provide an assay system where it was possible to go forward without the addition of exogenous MIF.

In the absence of any such suggestion, it is respectfully requested to acknowledge novelty and inventive step of the claims on file over the cited prior art documents.

positive septic shock. Anti-MIF antibodies were suggested as a means of therapy to modulate cytokine production in septic shock and other inflammatory disease states.

US 6,645,493 discloses monoclonal anti-MIF antibodies derived from hybridoma cells, which neutralize the biological activity of MIF. It could be shown in an animal model that these mouse-derived anti-MIF antibodies had a beneficial effect in the treatment of endotoxin-induced shock.

US 200310235584 discloses methods of preparing high affinity antibodies to MIF in animals in which the MIF gene has been homozygously knocked-out.

Glycosylation-inhibiting factor (GIF) is a protein described by Galat et al. (Eur. J. Biochem, 1994, 224, 417-21). MIF and GIF are now recognized to be identical. Watarai et al. (PNAS 2000, 97, 13251-6) described polyclonal antibodies binding to different GIF epitopes to identify the biochemical nature of the posttranslational modification of GIF in Ts cells. Watarai et al, *supra*, reported that GIF occurs in different conformational isoforms *in vitro*. One type of isomer occurs by chemical modification of a single cysteine residue. The chemical modification leads to conformational changes within the GIF protein.

In order to test the potency of anti-MIF antibodies a reliable and easy-to-use assay needs to be provided. It is of paramount importance, both for diagnostic and therapeutic purposes, to be able to define a given anti-MIF antibody-sample as to its potency. Without a clear indication of the respective potency of an anti-MIF antibody, the same cannot be used for either diagnosis or therapy.

Robust bioassays for potency assessment of MIF related drugs, in particular of anti-MIF antibodies, are not known so far.

Although it was shown in earlier assay formats that the MIF protein exhibits chemokine-like functions and that these functions can be blocked by anti-MIF antibodies (e.g. Bernhagen et al., Nature Medicine, 2007), no robust MIF bioassay based on the inhibition of autocrine-induced cell migration of e.g. monocytic cells has been established and qualified so far.

Thus, the object of the present invention is the provision of an assay which can determine the potency of anti-MIF antibodies. Preferably, this assay and respective assay method should be capable of providing highly sensitive and reproducible results and should at the same time be easy-to-use.

The present inventors set out to investigate how this goal could be achieved and have thus accomplished the present invention.

SUMMARY ~~OF THE INVENTION~~

The present ~~invention~~disclosure is directed to a highly sensitive, reproducible and easy to use - assay to determine the potency of anti-MIF antibodies, in particular anti-oxMIF antibodies.

The inventive assay is a cell migration assay, which is defined ~~as follows:~~below.

~~1.~~In one aspect, there is provided an ~~A~~assay method for determining the potency of anti-MIF antibodies, wherein a cell migration is performed, wherein the assay method comprises the following steps:

- providing cells, which are capable of migration, in a first part of a device, wherein the cells express MIF,

- adding the anti-(ox)MIF antibody to be determined to a second part of the device, which is configured to be in a connection with the first part of the device which allows cell migration and diffusion, and
- calculating inhibition of migration.

In one aspect, there is provided an assay kit for determining the potency of anti-(ox)MIF-antibodies, comprising all reagents necessary to carry out the assay method as defined herein, preferably

- cells which express MIF
- antibody dilution buffer (e.g. glycine buffer)
- migration medium, and/or
- Two chamber cell migration system.

In one aspect, there is provided a pharmaceutical or diagnostic composition, comprising anti-(ox)MIF antibodies, wherein the anti-(ox)MIF antibodies are characterized in that they have undergone a potency determination, as defined herein.

"Potency" in this context is a measure of drug activity expressed in terms of the amount required to produce an effect of given intensity. It is preferably expressed as an IC_{50} value (half maximal inhibition).

The assay of the present ~~invention~~disclosure is based on the principle of inhibiting autocrine chemotactic actions of (ox)MIF and thereby inhibiting migration of cells which express MIF, in particular oxMIF, preferably on their surface, e.g. certain monocytic cells, like U937 monocytic cells. The cells of the present ~~invention~~disclosure can express MIF endogenously or can be manipulated to recombinantly express MIF. The MIF needs to be expressed as oxMIF/converted to oxMIF. In a preferred embodiment the oxMIF is expressed on the surface of the cells, though this is not necessarily a key feature of this ~~invention~~disclosure.

The present assay is to be differentiated from a typical chemotaxis assay (as referred to in scientific literature) which is based on a directed migration of cells towards a chemoattractant gradient. This chemoattractant gradient according to the prior art would have been (ox)MIF, added to the device in addition to the cells. The present inventors could surprisingly show that it is possible to provide an assay using cells which express MIF and that it was thus possible to provide an assay without addition of a further chemoattractant, i.e. without addition of (ox) MIF. These findings were particularly advantageous as the resultant assay is ox-MIF-diffusion independent, i.e. diffusion over time will not interfere with the results, and thus the assay is not as time-sensitive as prior art assays. Anti-(ox)MIF antibodies are capable of inhibiting the pro-migratory (ox)MIF functions on cells expressing (ox)MIF, thereby inhibiting random cell migration ("chemokinesis") (rather than inhibiting cell chemotaxis as referred to in scientific literature).

The present ~~invention~~disclosure is also further explained by the attached figures.

Figure 1: General set-up of the present assay method.

Figure 2: FACS data of oxMIF on U937 monocytic cells

Figure 3: Exemplary figure showing two migration inhibition curves of U937 (human monocytes) and NR8383 (rat macrophages) towards different concentrations of RAM9 (anti-oxMIF antibody; logarithmic scale). FACS plot shows the binding of RAM9 to the cellular surface of these cells (black line; thin line: isotype control antibody)

DETAILED DESCRIPTION OF THE INVENTION

The invention is characterized, in part, by the following items:

1. Assay method for determining the potency of anti-MIF antibodies, wherein a cell migration is performed, wherein the assay method comprises the following steps:
   - providing cells, which are capable of migration, in a first part of a device, wherein the cells express MIF,