

Effect of the IMT Matcher benchtop imager light on mouse embryo development

REPORT REQUESTED BY IMT INTERNATIONAL

embryotools 

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1 Objective

To test whether the IMT Matcher benchtop imager light affects the embryo development. For this purpose, five mouse embryo assays (MEAs) were performed by over-exposing the embryos to the light source and then following embryo development and quality monitored up to day 6.

2 Imager settings

To have the maximum light outputs from the imager, the following imager settings were selected:

- Illumination On
- Normal Illumination
- Aiming On
- High Aiming Brightness

3 Experimental protocol

One printed Matcher barcode label (Ref: LBL020) was stuck on each culture dish. Culture dishes were prepared with previously tested culture medium in triplicate and equilibrated overnight prior to use (**Fig.1**).



Fig. 1. Culture dishes set-up.

Fresh 1-cell stage mouse embryos were collected from F1 hybrid females (B6/CBA) crossed with males from the same genetic background and washed thoroughly and cultured in labelled dishes.

Embryos were exposed to the imager light for 3 minutes every 24 hours (**Fig.2,3**). The light exposure protocol applied was the following:

- Step 1. Embryos were fully exposed to the imager light for 1.5 minutes.
- Step 2. Immediately after, the label on the dish was scanned for 1.5 minutes (50 beeps).

Note. With this procedure, embryos are exposed to the maximum amount of light (step 1) but at the same time a hypothetical effect of the light in the adhesive can be detected (step 2). Both light effects can be potentially harmful for embryo development and in consequence they were evaluated.

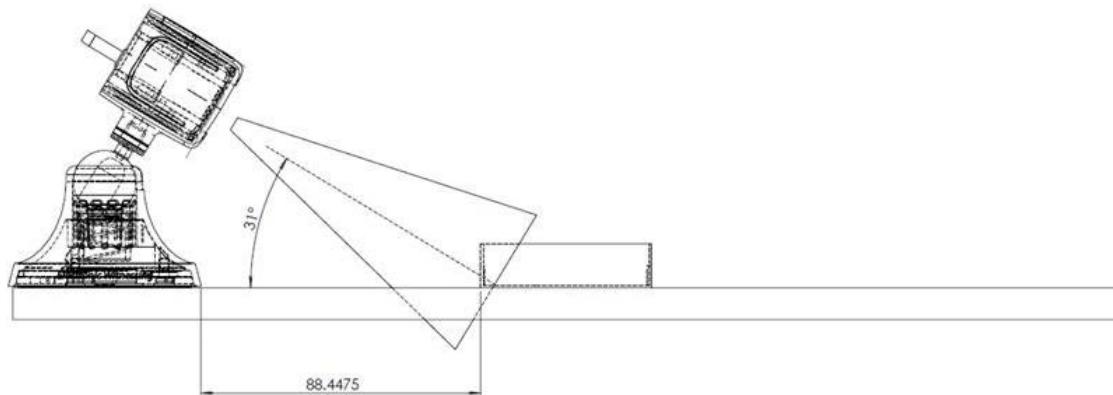


Fig. 2. Illustration of the optimum distance between the culture dishes and the imager for scanning barcode labels.

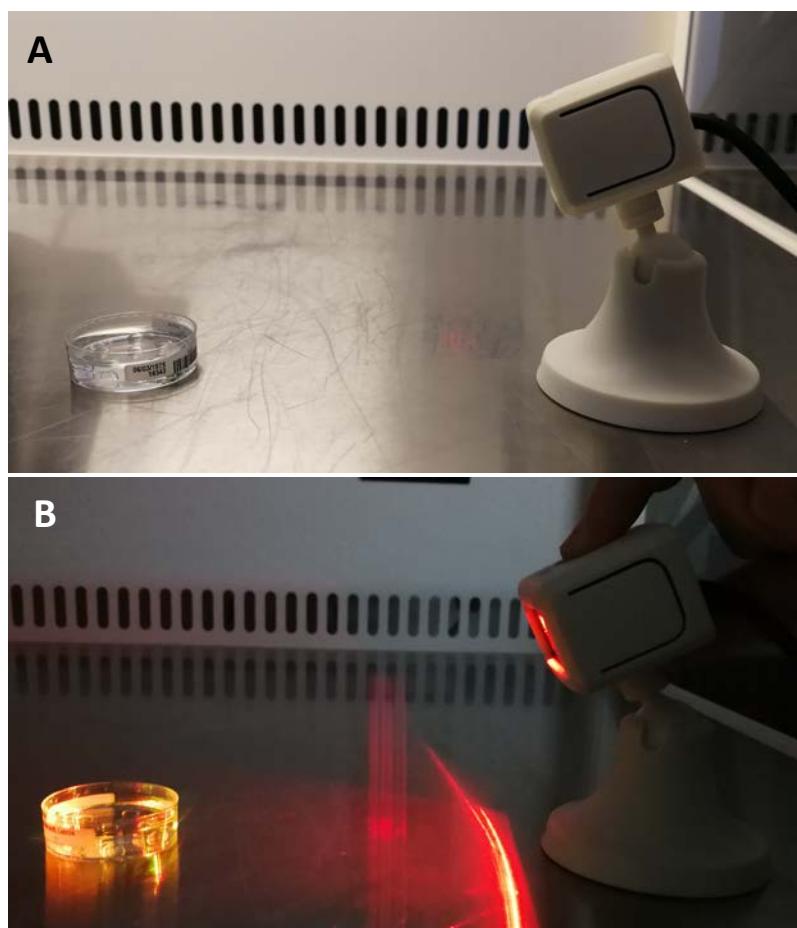


Fig. 3. Culture dishes exposure to the imager light beam. A) Light beam OFF. B) Light beam ON.

Embryo development was followed every 24 hours up to day 6, when the expanded blastocyst rate (EBR) and the good quality rate (GQR) was assessed.

4 Results

Five MEAs were performed fulfilling acceptance criteria.

Replica	Experiment code	n	2-cell stage rate n (%)	Day 6 Expanded blastocyst rate n (%)	Day 6 Good quality rate n (%)
R1	EXP.012.080.2019	21	21 (100)	21 (100)	21 (100)
R2	EXP.012.081.2019	21	21 (100)	19 (90.5)	19 (100)
R3	EXP.012.082.2019	21	21 (100)	20 (95.2)	19 (95.0)
R4	EXP.012.083.2019	21	21 (100)	20 (95.2)	20 (100)
R5	EXP.012.084.2019	21	21 (100)	21 (100)	17 (81.0)
TOTAL	-	105	105 (100)	101 (96.2)	96 (95.0)

Embryo developmental rates of control groups:

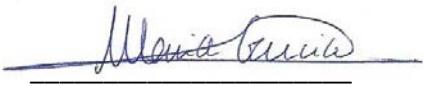
Control	n	2-cell stage rate n (%)	Day 6 Expanded blastocyst rate n (%)	Day 6 Good quality rate n (%)
1	15	15 (100)	14 (93.3)	13 (92.9)
2	15	15 (100)	15 (100)	13 (86.7)
TOTAL	30	30 (100)	29 (96.7)	26 (89.7)

Note. See the complete QC Certificates of Analysis for each replica in Annex I.

5 Conclusions

- A total of 105 mouse embryos were exposed to the imager light and cell degeneration was not shown in any of the replicas.
- No differences were found between the test and control groups with regards to the expanded blastocyst formation rate and good quality rates after exposing the test group to the imager light daily.
- We conclude that the imager light does not seem affect the mouse embryo development.

Barcelona, 8th February 2019



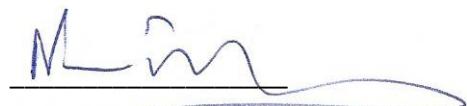
Maria Garcia, MSc

Embryologist



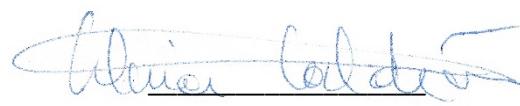
Enric Mestres, MSc

Lab manager



Nuno Costa-Borges, PhD

Scientific Director



Gloria Calderón, PhD

Director

Annex I. QC Certificates of Analysis

QC



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Phone: +34 934 497 198

Certificate of Analysis

REQUESTED BY: Embryotools, S.L. (Avda. Dr. Marañón, 8, Barcelona 08028 BARCELONA 724)

ASSAY REQUESTED BY CUSTOMER: EXP - Experiment

OPERATION PROCEDURE: SOP-MEA-00/WI-MEA-05

TYPE OF ASSAY: Direct

INTERNAL NUMBER: EXP.012.080.2019

DATE: 15/01/2019 - 20/01/2019

DESCRIPTION OF TEST PRODUCT: Study of the IMT Matcher benchtop imager light on mouse embryo development, R1 (IMT International)

REF: N/A

LOT NUMBER: N/A

EXP. DATE: N/A

PROTOCOL:

One label was stuck on each culture dish. Culture dishes were prepared with previously tested culture medium in triplicate and equilibrated overnight prior to use. Fresh 1-cell stage mouse embryos were collected from F1 hybrid females (B6/CBA) crossed with males from the same genetic background and washed thoroughly and cultured in labelled dishes. Embryos were exposed to the imager light for 3 minutes every 24 hours (as explained in the project plan). Control group was prepared following the same set-up and conditions, and embryos cultured in parallel without the imager light exposure. Embryo development of test and control group was followed every 24 h up to day 6 and photos were taken and included in this report (annex I).

CONTROL AND TEST ASSAY RESULTS:

Embryo developmental rates of control and tested group.

Embryo development rates					
	n	Day 2 Two-cell stage n (%)	Day 6 Expanded blastocyst stage n (%)	Good Quality (morphology) Blastocysts n (%)	Result
Control	15	15 (100)	14 (93.33)	13 (92.86)	Passed*
Study of the IMT Matcher benchtop imager light on mouse embryo development, R1 (IMT International) (Lot:N/A)	21	21 (100)	21 (100)	21 (100)	Passed*

SUMMARY OF OBSERVATIONS: All test and control embryos were selected randomly from a common pool and cultured at 37.3°C with a tri-gas atmosphere with optimal %CO₂ and %O₂. Embryotools acceptance criteria for this standard test is that more than 80% of mouse embryos develop to the expanded blastocyst stage and pass a visual morphological examination of the inner cell mass (ICM) and trophectoderm (TE) cells. The results of this assay refer to the items tested.

* More than 80% of the test group embryos developed to the expanded blastocyst stage within 6 days, fulfilling acceptance criteria for this test.

Nuno Costa-Borges, PhD

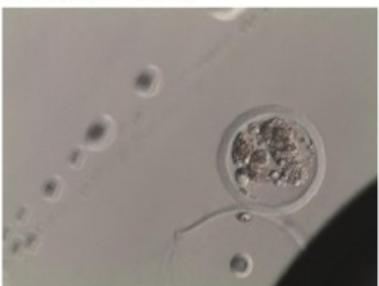
Scientific Director

Gloria Calderón, PhD

Quality Assurance

Annex I Control

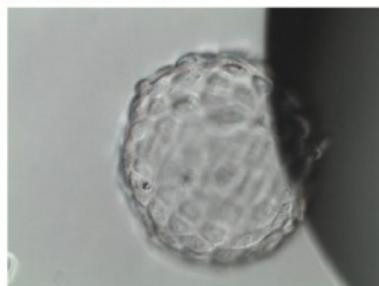
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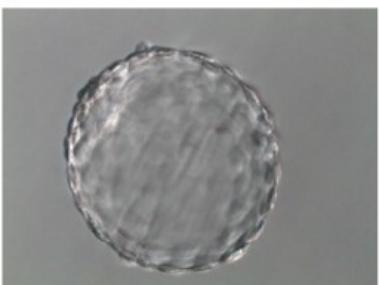
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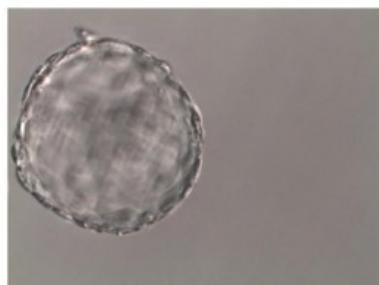
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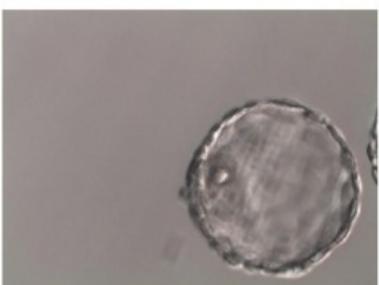
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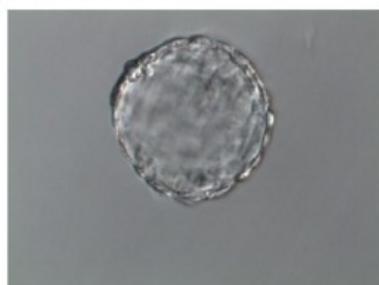
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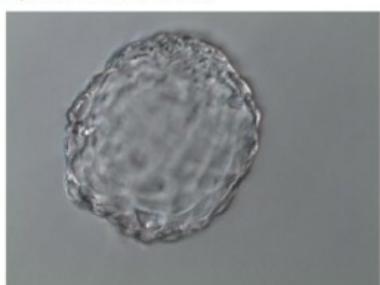
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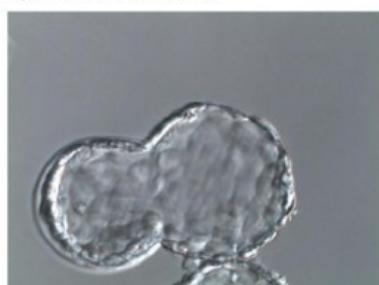
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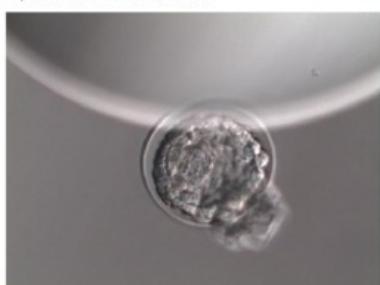
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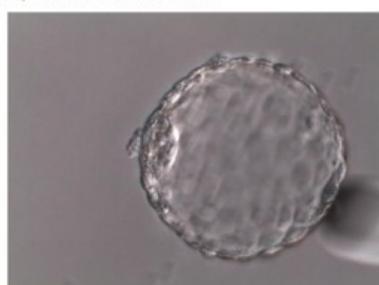
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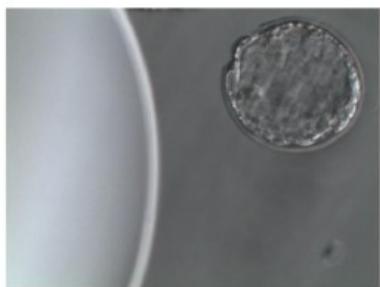
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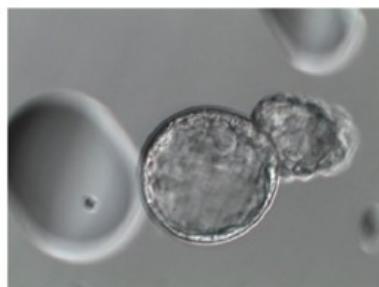
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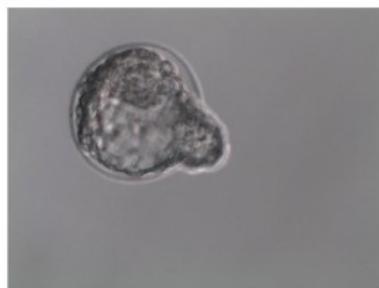
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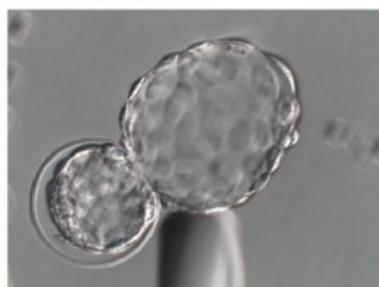
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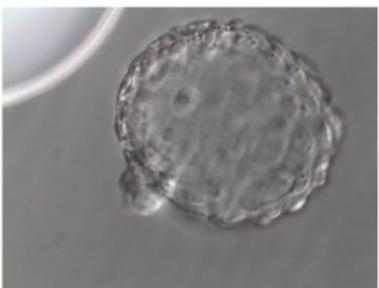
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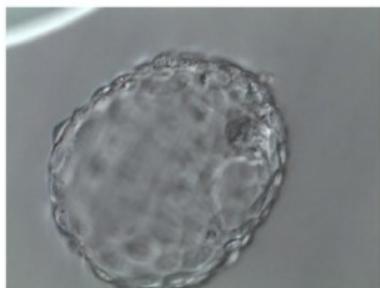
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QC



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Certificate of Analysis

REQUESTED BY: Embryotools, S.L. (Avda. Dr. Marañón, 8, Barcelona 08028 BARCELONA 724)

ASSAY REQUESTED BY CUSTOMER: EXP - Experiment

OPERATION PROCEDURE: SOP-MEA-00/WI-MEA-05

TYPE OF ASSAY: Direct

INTERNAL NUMBER: EXP.012.081.2019

DATE: 15/01/2019 - 20/01/2019

DESCRIPTION OF TEST PRODUCT: Study of the IMT Matcher benchtop imager light on mouse embryo development, R2 (IMT International)

REF: N/A

LOT NUMBER: N/A

EXP. DATE: N/A

PROTOCOL:

One label was stuck on each culture dish. Culture dishes were prepared with previously tested culture medium in triplicate and equilibrated overnight prior to use. Fresh 1-cell stage mouse embryos were collected from F1 hybrid females (B6/CBA) crossed with males from the same genetic background and washed thoroughly and cultured in labelled dishes. Embryos were exposed to the imager light for 3 minutes every 24 hours (as explained in the project plan). Control group was prepared following the same set-up and conditions, and embryos cultured in parallel without the imager light exposure. Embryo development of test and control group was followed every 24 h up to day 6 and photos were taken and included in this report (annex I).

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Embryo developmental rates of control and tested group.

Embryo development rates					
	n	Day 2 Two-cell stage n (%)	Day 6 Expanded blastocyst stage n (%)	Good Quality (morphology) Blastocysts n (%)	Result
Control	15	15 (100)	14 (93.33)	13 (92.86)	Passed*
Study of the IMT Matcher benchtop imager light on mouse embryo development, R2 (IMT International) (Lot:N/A)	21	21 (100)	19 (90.48)	19 (100)	Passed*

SUMMARY OF OBSERVATIONS: All test and control embryos were selected randomly from a common pool and cultured at 37.3°C with a tri-gas atmosphere with optimal %CO₂ and %O₂. Embryotools acceptance criteria for this standard test is that more than 80% of mouse embryos develop to the expanded blastocyst stage and pass a visual morphological examination of the inner cell mass (ICM) and trophectoderm (TE) cells. The results of this assay refer to the items tested.

* More than 80% of the test group embryos developed to the expanded blastocyst stage within 6 days, fulfilling acceptance criteria for this test.

Nuno Costa-Borges, PhD

Scientific Director

Gloria Calderón, PhD

Quality Assurance

Annex I Control

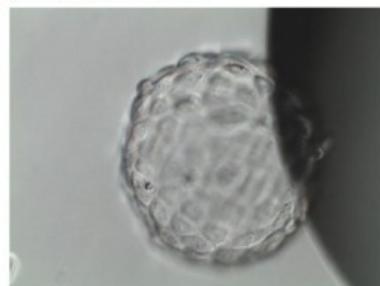
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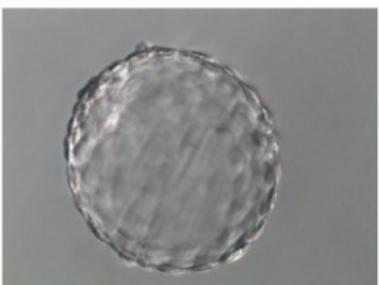
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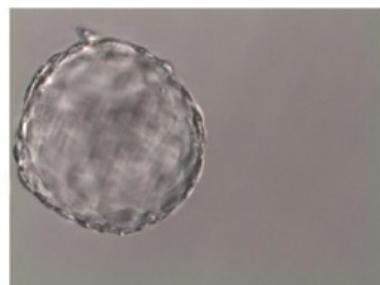
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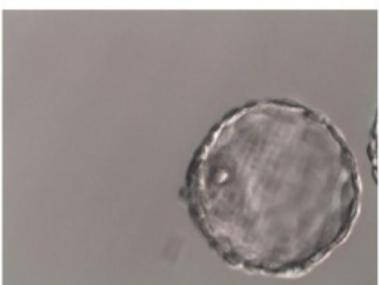
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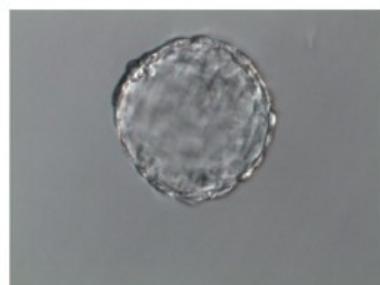
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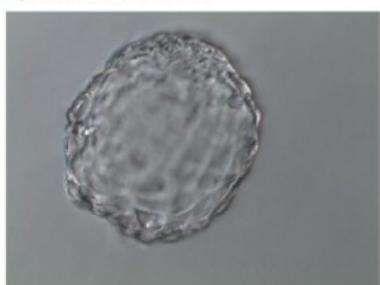
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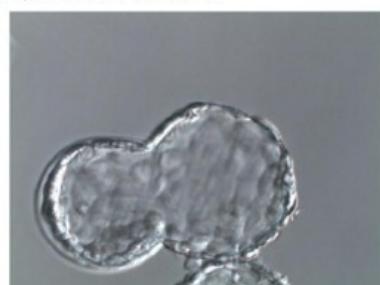
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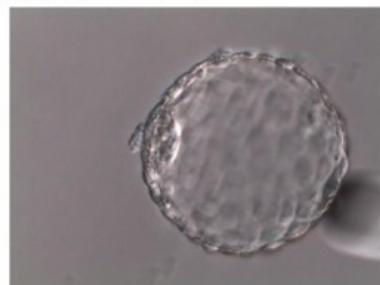
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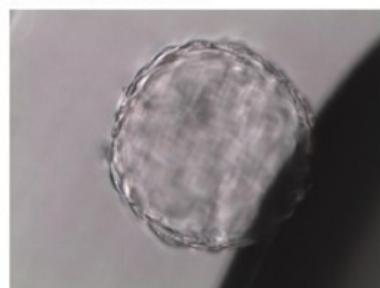
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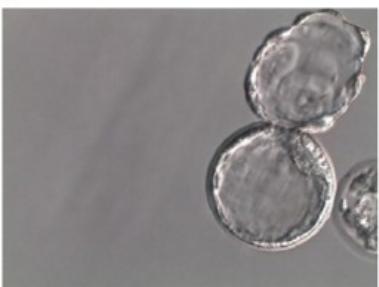
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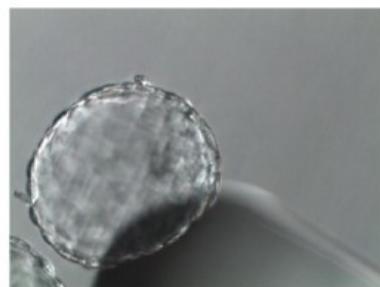
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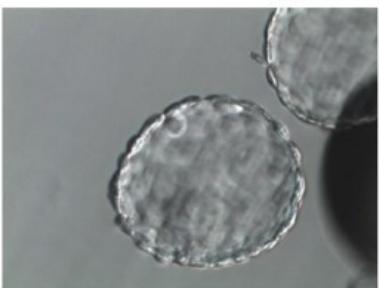
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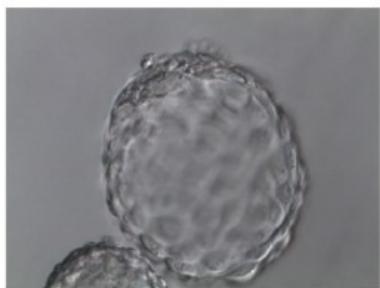
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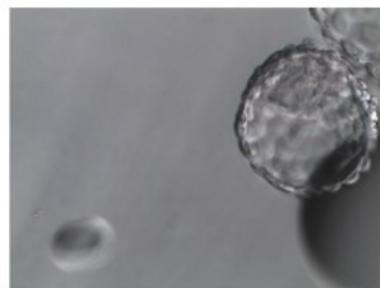
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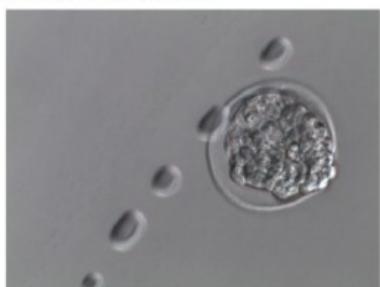
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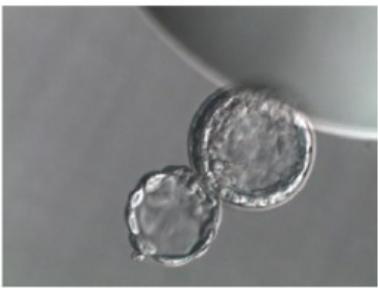
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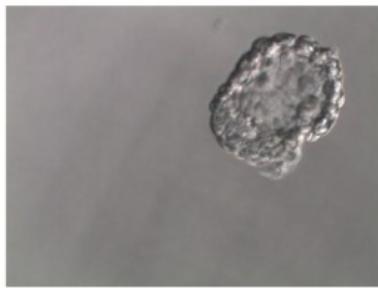
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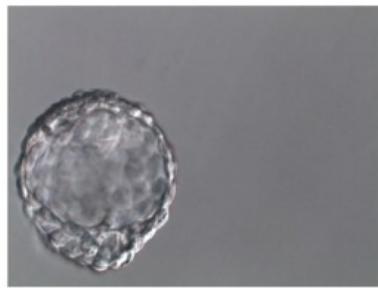
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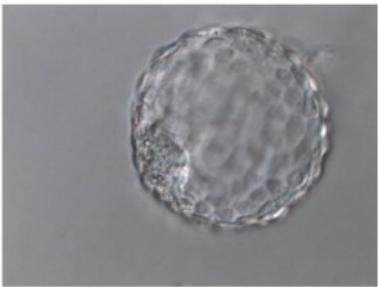
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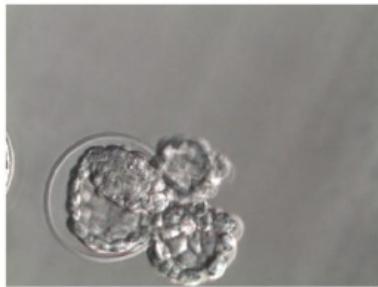
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QC



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Phone: +34 934 497 198

Certificate of Analysis

REQUESTED BY: Embryotools, S.L. (Avda. Dr. Marañón, 8, Barcelona 08028 BARCELONA 724)

ASSAY REQUESTED BY CUSTOMER: EXP - Experiment

OPERATION PROCEDURE: SOP-MEA-00/WI-MEA-05

TYPE OF ASSAY: Direct

INTERNAL NUMBER: EXP.012.082.2019

DATE: 15/01/2019 - 20/01/2019

DESCRIPTION OF TEST PRODUCT: Study of the IMT Matcher benchtop imager light on mouse embryo development, R3 (IMT International)

REF: N/A

LOT NUMBER: N/A

EXP. DATE: N/A

PROTOCOL:

One label was stuck on each culture dish. Culture dishes were prepared with previously tested culture medium in triplicate and equilibrated overnight prior to use. Fresh 1-cell stage mouse embryos were collected from F1 hybrid females (B6/CBA) crossed with males from the same genetic background and washed thoroughly and cultured in labelled dishes. Embryos were exposed to the imager light for 3 minutes every 24 hours (as explained in the project plan). Control group was prepared following the same set-up and conditions, and embryos cultured in parallel without the imager light exposure. Embryo development of test and control group was followed every 24 h up to day 6 and photos were taken and included in this report (annex I).

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Control	15	15 (100)	14 (93.33)	13 (92.86)	Passed*
Study of the IMT Matcher benchtop imager light on mouse embryo development, R3 (IMT International) (Lot:N/A)	21	21 (100)	20 (95.24)	19 (95)	Passed*

SUMMARY OF OBSERVATIONS: All test and control embryos were selected randomly from a common pool and cultured at 37.3°C with a tri-gas atmosphere with optimal %CO₂ and %O₂. Embryotools acceptance criteria for this standard test is that more than 80% of mouse embryos develop to the expanded blastocyst stage and pass a visual morphological examination of the inner cell mass (ICM) and trophectoderm (TE) cells. The results of this assay refer to the items tested.

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Nuno Costa-Borges, PhD

Scientific Director

Gloria Calderón, PhD

Quality Assurance

Annex I Control

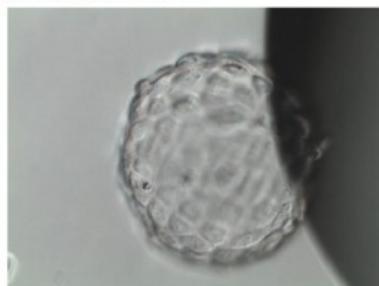
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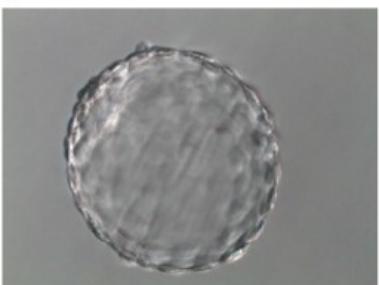
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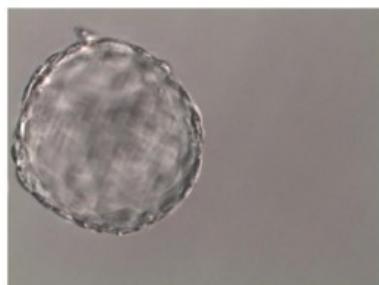
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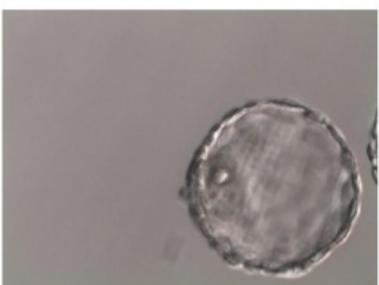
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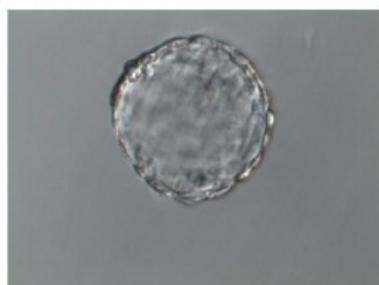
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#8, 20/01/2019 10:51:05



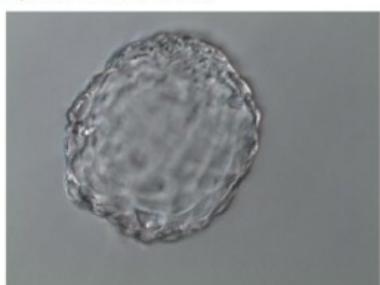
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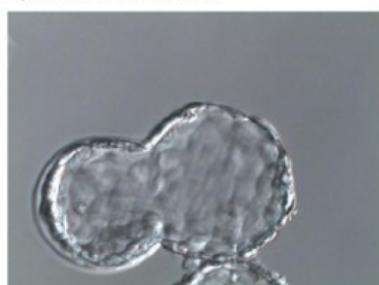
#10, 20/01/2019 10:51:29



#11, 20/01/2019 10:51:32



#12, 20/01/2019 10:51:50



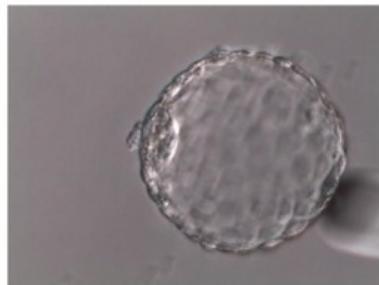
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#14, 20/01/2019 10:52:12

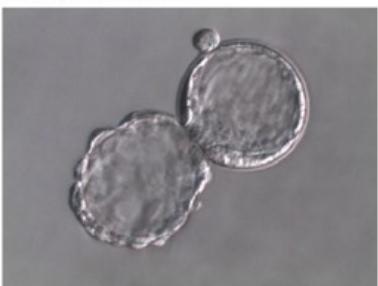


#15, 20/01/2019 10:52:14



Study of the IMT Matcher benchtop imager light on mouse embryo development R3 (IMT International)
(REF: N/A ; Lot: N/A)

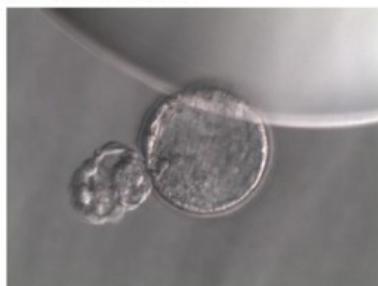
#1, 20/01/2019 11:40:05



#2, 20/01/2019 11:40:10



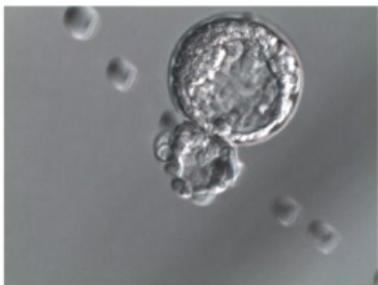
#3, 20/01/2019 11:40:14



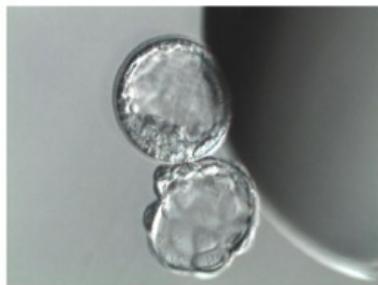
#4, 20/01/2019 11:40:38



#5, 20/01/2019 11:40:41



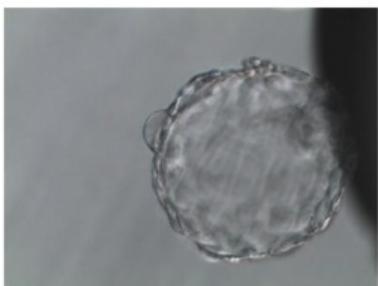
#6, 20/01/2019 11:40:55



#7, 20/01/2019 11:41:00



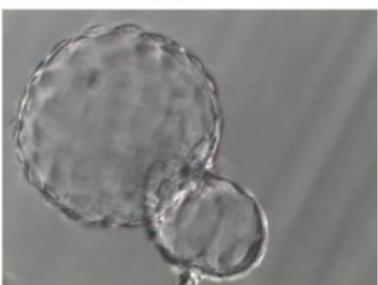
#8, 20/01/2019 11:41:14



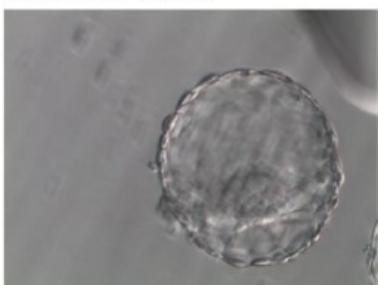
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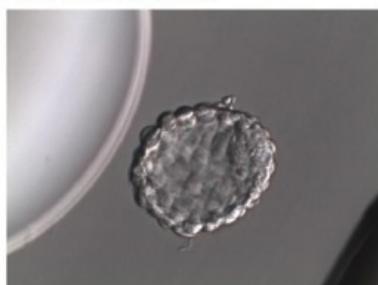
#10, 20/01/2019 11:41:36



#11, 20/01/2019 11:41:40



#12, 20/01/2019 11:41:58



#13, 20/01/2019 11:42:02



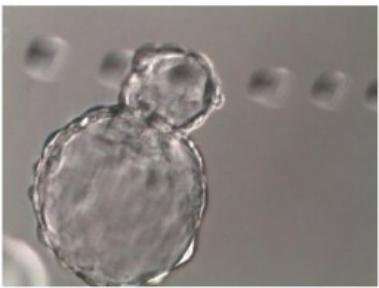
#14, 20/01/2019 11:42:23



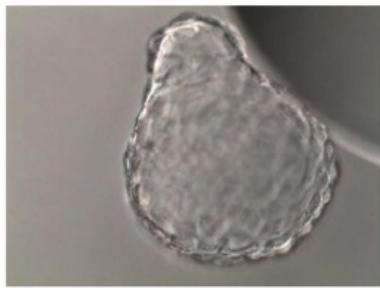
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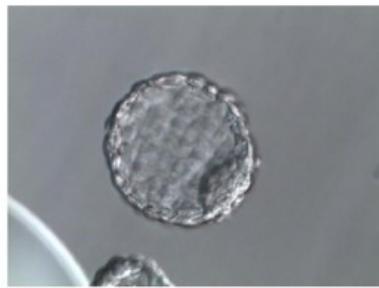
#16, 20/01/2019 11:42:43



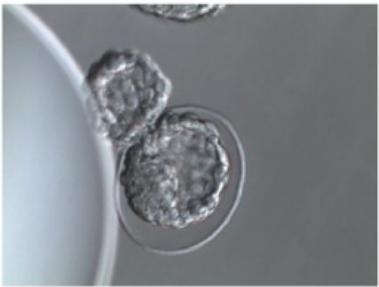
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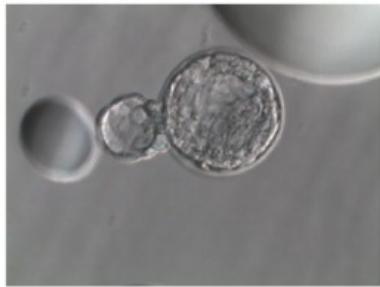
#18, 20/01/2019 11:42:59



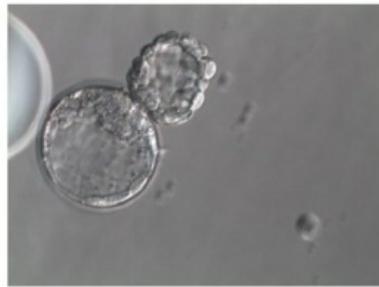
#19, 20/01/2019 11:43:01



#20, 20/01/2019 11:43:15



#21, 20/01/2019 11:43:18



QC



Parc Científic de Barcelona // Avda. Doctor Marañón, 8
08028 Barcelona, Spain
NIF B66034612 // info@embryotools.com
Phone: +34 934 497 198

Certificate of Analysis

REQUESTED BY: Embryotools, S.L. (Avda. Dr. Marañón, 8, Barcelona 08028 BARCELONA 724)

ASSAY REQUESTED BY CUSTOMER: EXP - Experiment

OPERATION PROCEDURE: SOP-MEA-00/WI-MEA-05

TYPE OF ASSAY: Direct

INTERNAL NUMBER: EXP.012.083.2019

DATE: 21/01/2019 - 26/01/2019

DESCRIPTION OF TEST PRODUCT: Study of the IMT Matcher benchtop imager light on mouse embryo development, R4 (IMT International)

REF: N/A

LOT NUMBER: N/A

EXP. DATE: N/A

PROTOCOL:

One label was stuck on each culture dish. Culture dishes were prepared with previously tested culture medium in triplicate and equilibrated overnight prior to use. Fresh 1-cell stage mouse embryos were collected from F1 hybrid females (B6/CBA) crossed with males from the same genetic background and washed thoroughly and cultured in labelled dishes. Embryos were exposed to the imager light for 3 minutes every 24 hours (as explained in the project plan). Control group was prepared following the same set-up and conditions, and embryos cultured in parallel without the imager light exposure. Embryo development of test and control group was followed every 24 h up to day 6 and photos were taken and included in this report (annex I).

CONTROL AND TEST ASSAY RESULTS:

Embryo developmental rates of control and tested group.

Embryo development rates					
	n	Day 2 Two-cell stage n (%)	Day 6 Expanded blastocyst stage n (%)	Good Quality (morphology) Blastocysts n (%)	Result
Control	15	15 (100)	15 (100)	13 (86.67)	Passed*
Study of the IMT Matcher benchtop imager light on mouse embryo development, R4 (IMT International) (Lot:N/A)	21	21 (100)	20 (95.24)	20 (100)	Passed*

SUMMARY OF OBSERVATIONS: All test and control embryos were selected randomly from a common pool and cultured at 37.3°C with a tri-gas atmosphere with optimal %CO₂ and %O₂. Embryotools acceptance criteria for this standard test is that more than 80% of mouse embryos develop to the expanded blastocyst stage and pass a visual morphological examination of the inner cell mass (ICM) and trophectoderm (TE) cells. The results of this assay refer to the items tested.

* More than 80% of the test group embryos developed to the expanded blastocyst stage within 6 days, fulfilling acceptance criteria for this test.

Nuno Costa-Borges, PhD

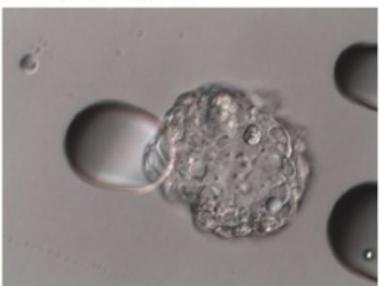
Scientific Director

Gloria Calderón, PhD

Quality Assurance

Annex I Control

#1, 26/01/2019 11:30:25



#2, 26/01/2019 11:30:28



#3, 26/01/2019 11:30:30



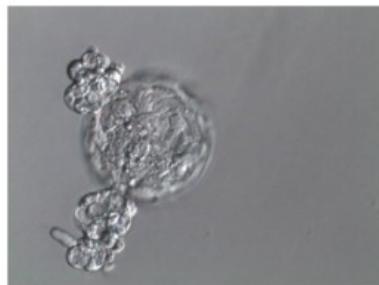
#4, 26/01/2019 11:30:54



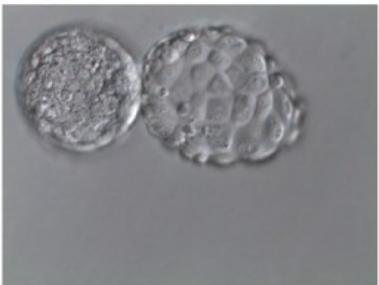
#5, 26/01/2019 11:30:56



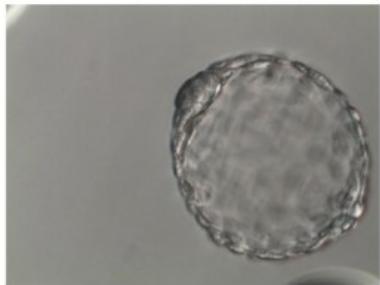
#6, 26/01/2019 11:31:07



#7, 26/01/2019 11:31:08



#8, 26/01/2019 11:31:23



#9, 26/01/2019 11:31:25



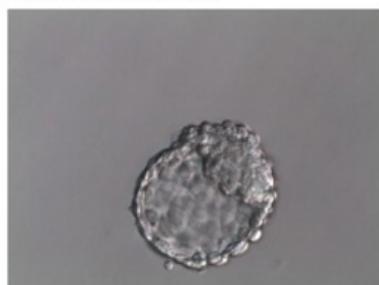
#10, 26/01/2019 11:31:36



#11, 26/01/2019 11:31:39



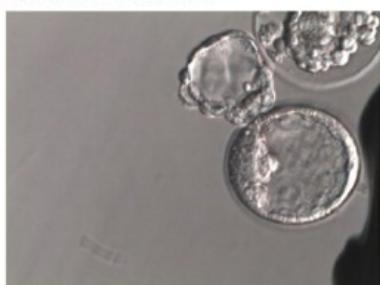
#12, 26/01/2019 11:31:49



#13, 26/01/2019 11:31:51



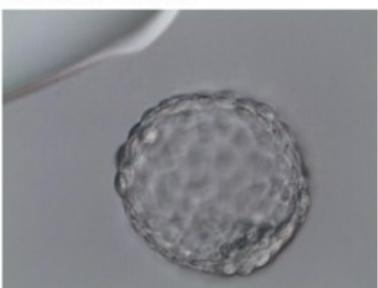
#14, 26/01/2019 11:32:04



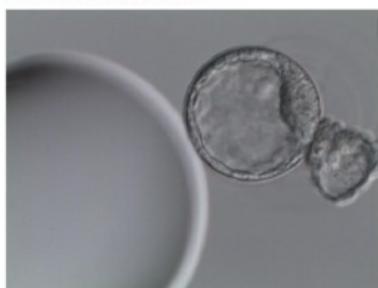
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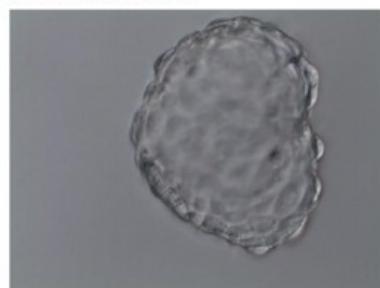
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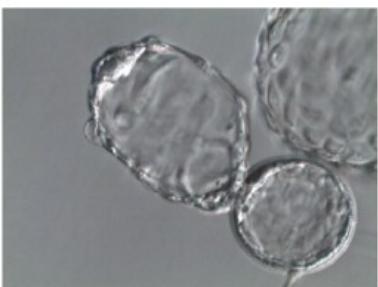
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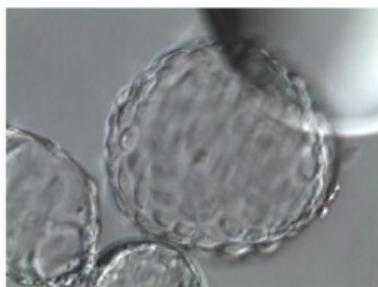
#3, 26/01/2019 11:21:47



#4, 26/01/2019 11:22:00



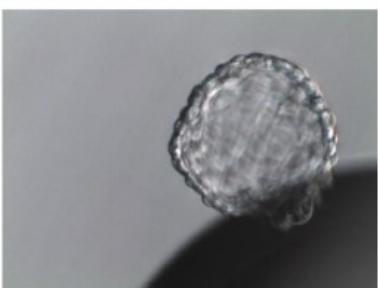
#5, 26/01/2019 11:22:01



#6, 26/01/2019 11:22:17



#7, 26/01/2019 11:22:18



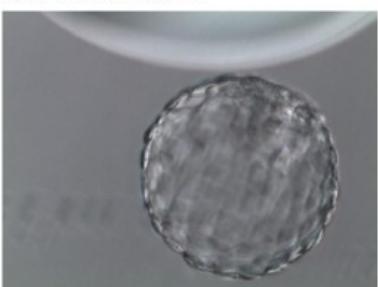
#8, 26/01/2019 11:22:32



#9, 26/01/2019 11:22:33



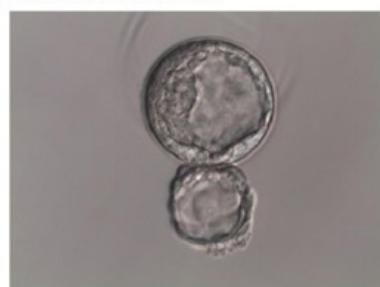
#10, 26/01/2019 11:22:39



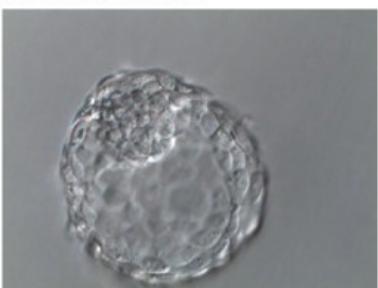
#11, 26/01/2019 11:22:42



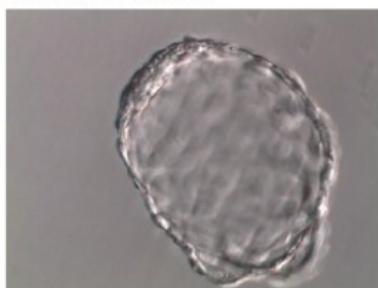
#12, 26/01/2019 11:26:13



#13, 26/01/2019 11:26:22



#14, 26/01/2019 11:26:34



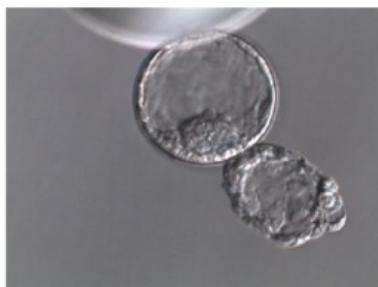
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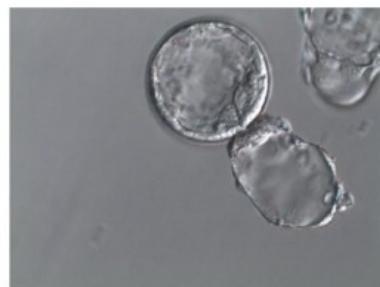
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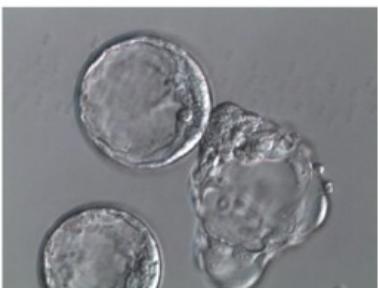
#17, 26/01/2019 11:26:51



#18, 26/01/2019 11:26:57



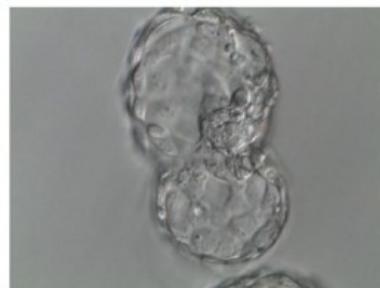
#19, 26/01/2019 11:26:59



#20, 26/01/2019 11:27:11



#21, 26/01/2019 11:27:13



QC



Parc Científic de Barcelona // Avda. Doctor Marañón, 8
08028 Barcelona, Spain
NIF B66034612 // info@embryotools.com
Phone: +34 934 497 198

Certificate of Analysis

REQUESTED BY: Embryotools, S.L. (Avda. Dr. Marañón, 8, Barcelona 08028 BARCELONA 724)

ASSAY REQUESTED BY CUSTOMER: EXP - Experiment

OPERATION PROCEDURE: SOP-MEA-00/WI-MEA-05

TYPE OF ASSAY: Direct

INTERNAL NUMBER: EXP.012.084.2019

DATE: 21/01/2019 - 26/01/2019

DESCRIPTION OF TEST PRODUCT: Study of the IMT Matcher benchtop imager light on mouse embryo development, R5 (IMT International)

REF: N/A

LOT NUMBER: N/A

EXP. DATE: N/A

PROTOCOL:

One label was stuck on each culture dish. Culture dishes were prepared with previously tested culture medium in triplicate and equilibrated overnight prior to use. Fresh 1-cell stage mouse embryos were collected from F1 hybrid females (B6/CBA) crossed with males from the same genetic background and washed thoroughly and cultured in labelled dishes. Embryos were exposed to the imager light for 3 minutes every 24 hours (as explained in the project plan). Control group was prepared following the same set-up and conditions, and embryos cultured in parallel without the imager light exposure. Embryo development of test and control group was followed every 24 h up to day 6 and photos were taken and included in this report (annex I).

CONTROL AND TEST ASSAY RESULTS:

Embryo developmental rates of control and tested group.

Embryo development rates					
	n	Day 2 Two-cell stage n (%)	Day 6 Expanded blastocyst stage n (%)	Good Quality (morphology) Blastocysts n (%)	Result
Control	15	15 (100)	15 (100)	13 (86.67)	Passed*
Study of the IMT Matcher benchtop imager light on mouse embryo development, R5 (IMT International) (Lot:N/A)	21	21 (100)	21 (100)	17 (80.95)	Passed*

SUMMARY OF OBSERVATIONS: All test and control embryos were selected randomly from a common pool and cultured at 37.3°C with a tri-gas atmosphere with optimal %CO₂ and %O₂. Embryotools acceptance criteria for this standard test is that more than 80% of mouse embryos develop to the expanded blastocyst stage and pass a visual morphological examination of the inner cell mass (ICM) and trophectoderm (TE) cells. The results of this assay refer to the items tested.

* More than 80% of the test group embryos developed to the expanded blastocyst stage within 6 days, fulfilling acceptance criteria for this test.

Nuno Costa-Borges, PhD

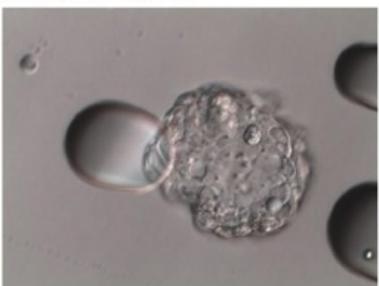
Scientific Director

Gloria Calderón, PhD

Quality Assurance

Annex I Control

#1, 26/01/2019 11:30:25



#2, 26/01/2019 11:30:28



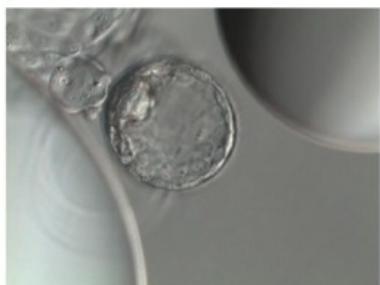
#3, 26/01/2019 11:30:30



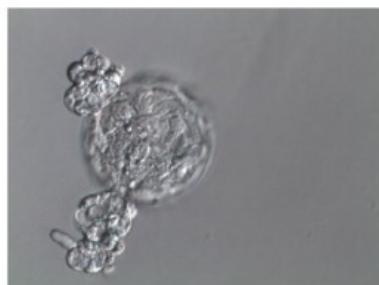
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#5, 26/01/2019 11:30:56



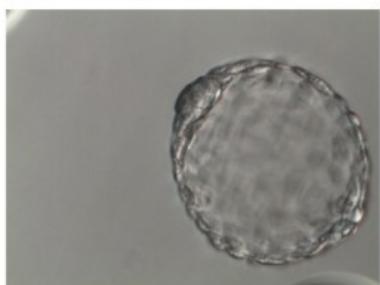
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#7, 26/01/2019 11:31:08



#8, 26/01/2019 11:31:23



#9, 26/01/2019 11:31:25



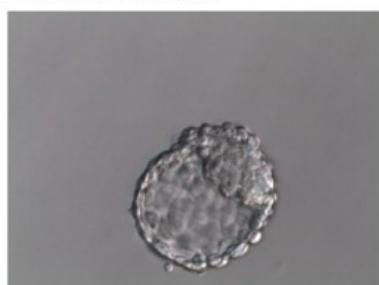
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#11, 26/01/2019 11:31:39



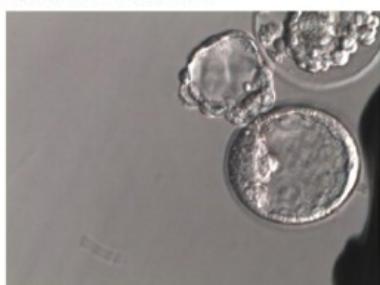
#12, 26/01/2019 11:31:49



#13, 26/01/2019 11:31:51



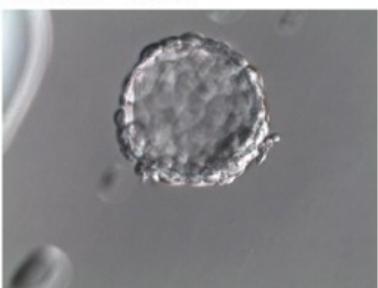
#14, 26/01/2019 11:32:04



#15, 26/01/2019 11:32:06



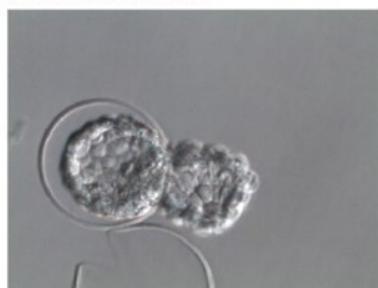
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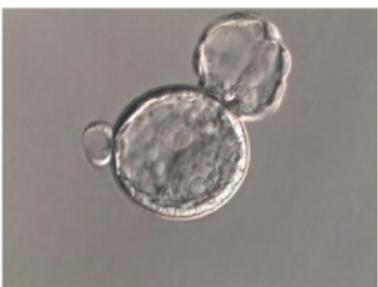
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#3, 26/01/2019 11:35:06



#4, 26/01/2019 11:35:26



#5, 26/01/2019 11:35:27



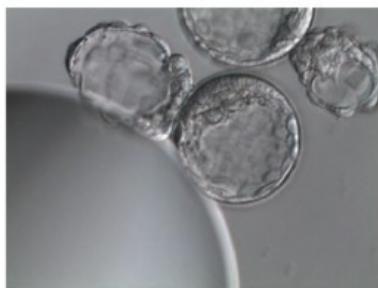
#6, 26/01/2019 11:35:40



#7, 26/01/2019 11:35:41



#8, 26/01/2019 11:35:53



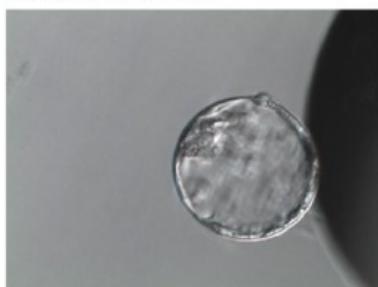
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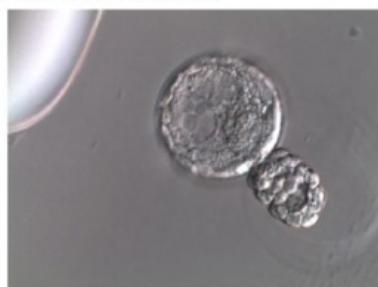
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#12, 26/01/2019 11:36:30



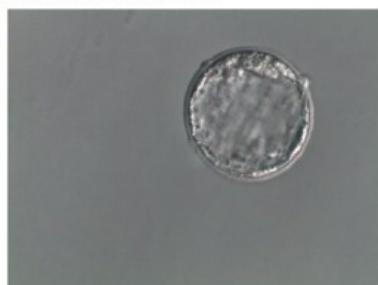
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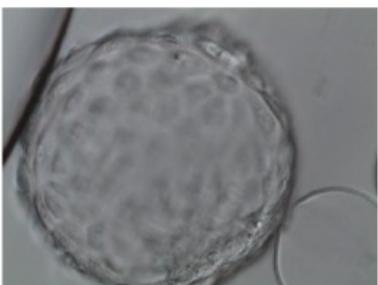
#14, 26/01/2019 11:36:41



#15, 26/01/2019 11:36:42



#16, 26/01/2019 11:36:54



#17, 26/01/2019 11:36:58



#18, 26/01/2019 11:37:08



#19, 26/01/2019 11:37:09



#20, 26/01/2019 11:37:22



#21, 26/01/2019 11:37:24

