



Validation of the G-185 incubator

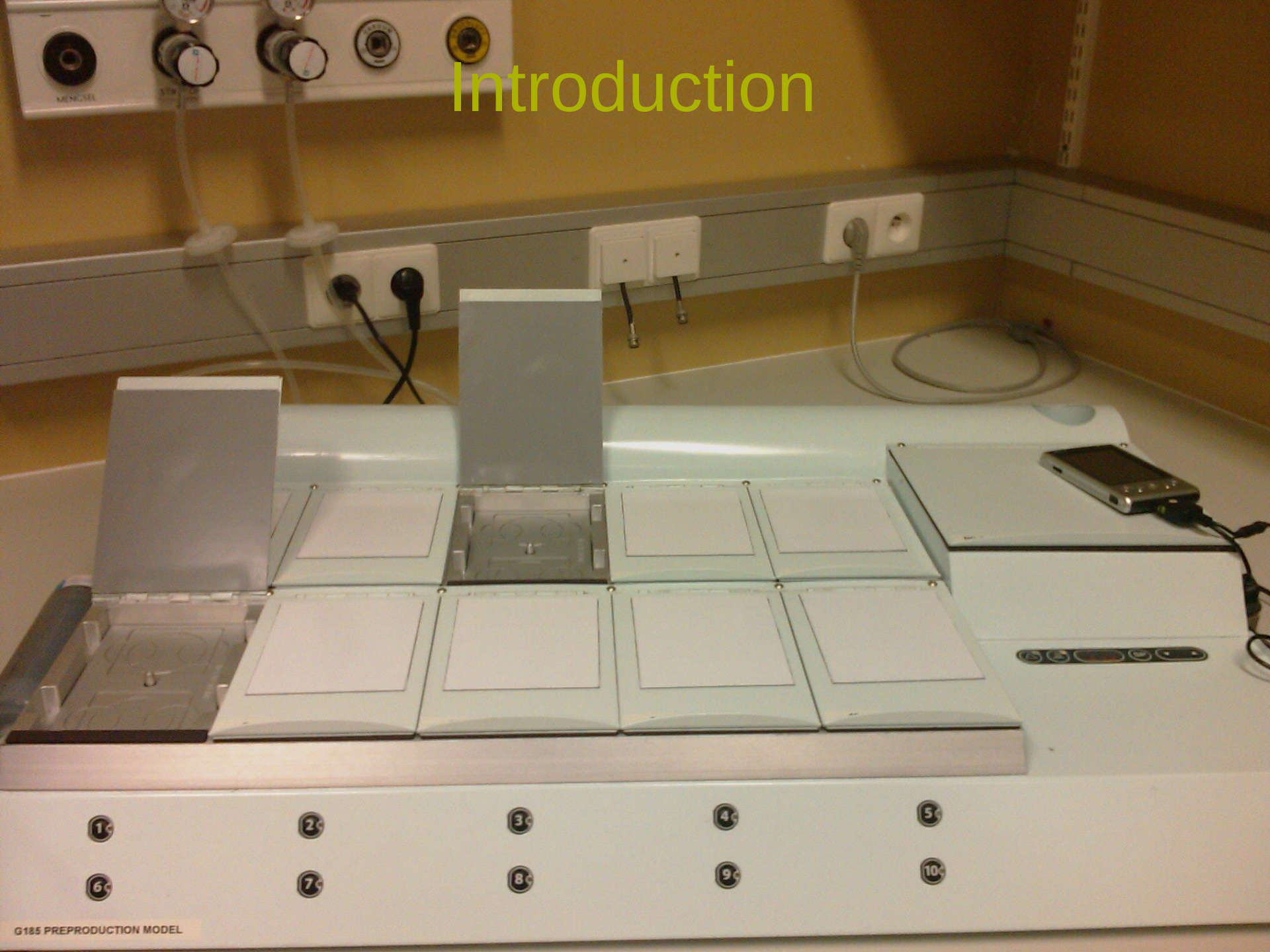
Ronny Janssens – Romy Souffreau

29/06/2009



Universitair Ziekenhuis Brussel

Introduction



1

2

3

4

5

6

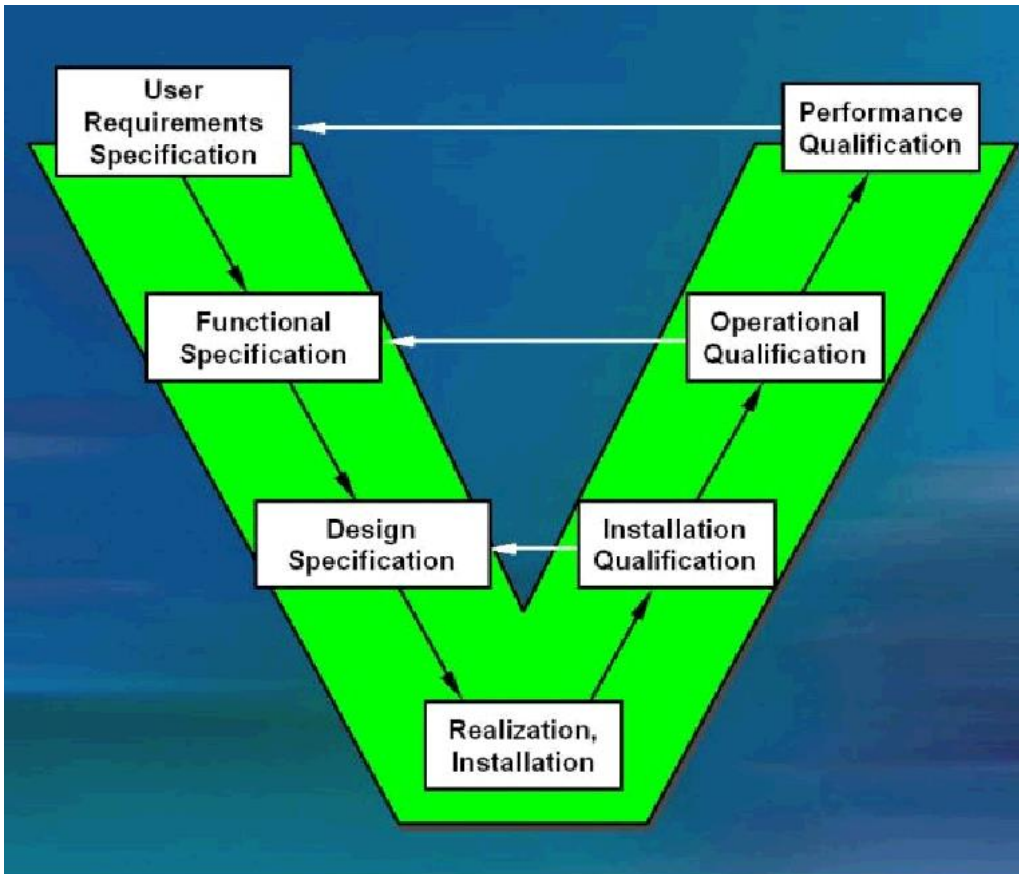
7

8

9

10

Validation



ISO 15189, §5.3.2

“Equipment shall be shown (upon installation and in routine use) to be capable of achieving the performance required and shall comply with specifications relevant to the examinations concerned”

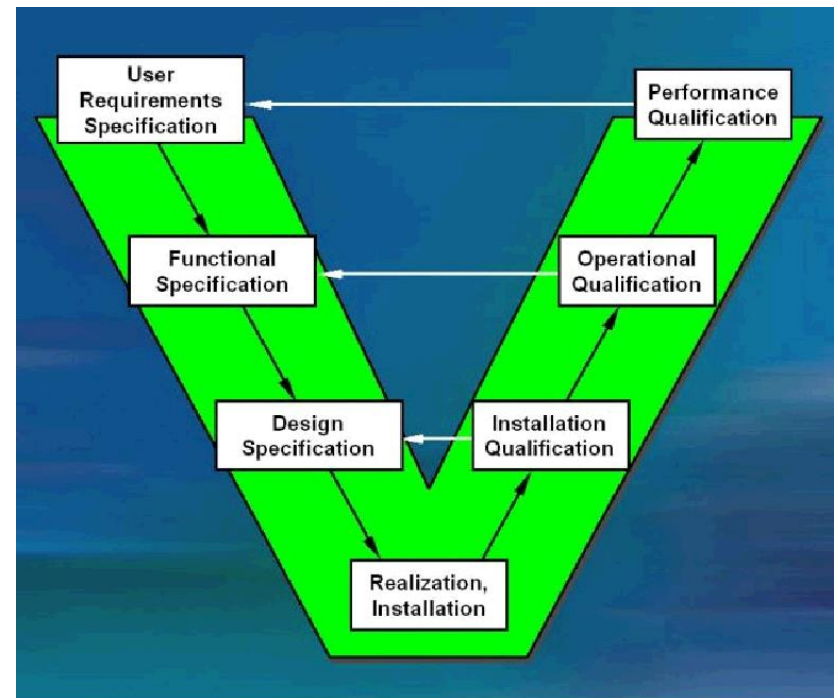
ISO 15189 §5.5.2

“The laboratory shall use only validated procedures for confirming that the examination procedures are suitable for the intended use”

- Technical validation
- Clinical validation
 - 3PN zygotes
 - Sibling oocyte study: G-185 vs Heracell240
 - Randomised Controlled Trial

Validation

- User requirements specifications
 - Use as safe incubator for IVF
 - Space saving alternative for standard incubators
- Functional specifications
 - Grow viable embryos
 - Good embryo quality
 - Easy to use
 - Alarm functions
- Design specifications
 - $37^{\circ}\text{C} \pm 0,5^{\circ}\text{C}$
 - $6\% \text{ CO}_2 \pm 0,5\% - 5\% \text{ O}_2 \pm 1\%$
 - Stable culture conditions
 - No humidification
 - Air quality (HEPA – VOC)



- **Technical validation**

- Pre production model (2007 - 2008)
- Final product (2008 - 2009)

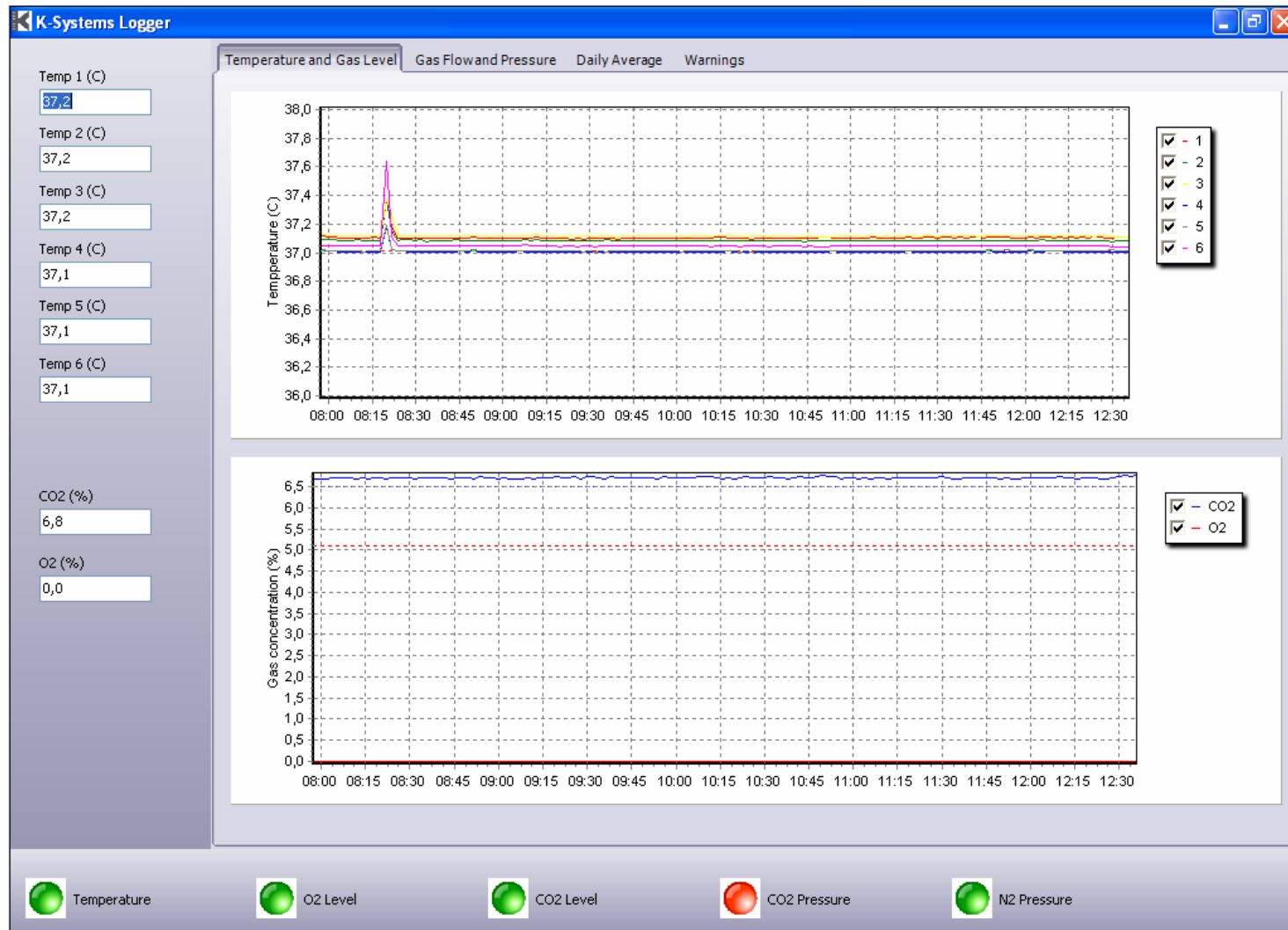
- **Clinical validation (2009)**

- 3PN zygotes
- Sibling oocyte study: G-185 vs Heracell240
- Randomised Controlled Trial (started 26/05/2009)

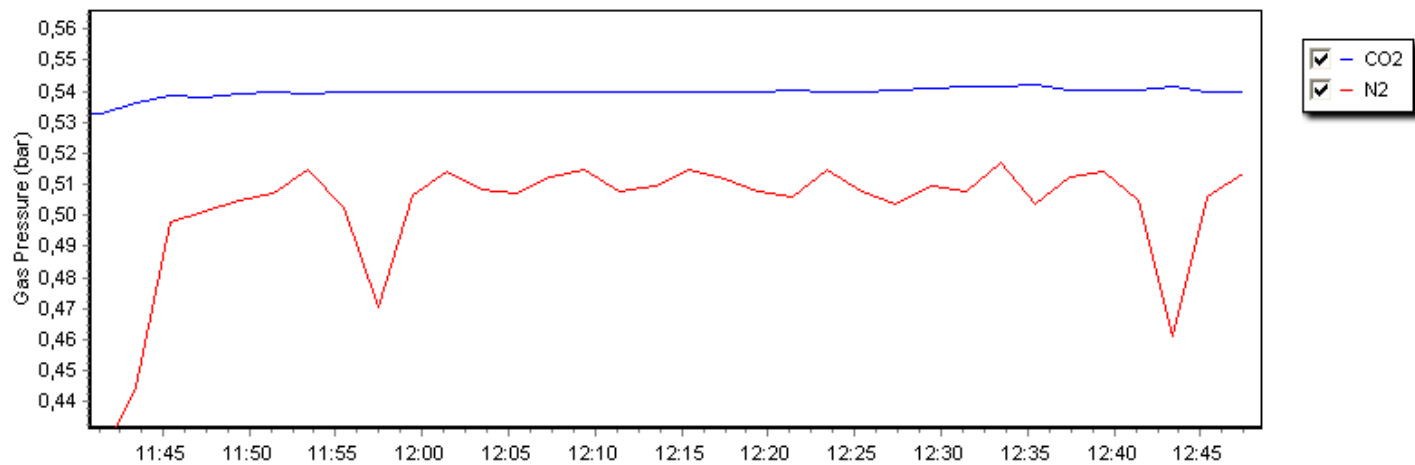
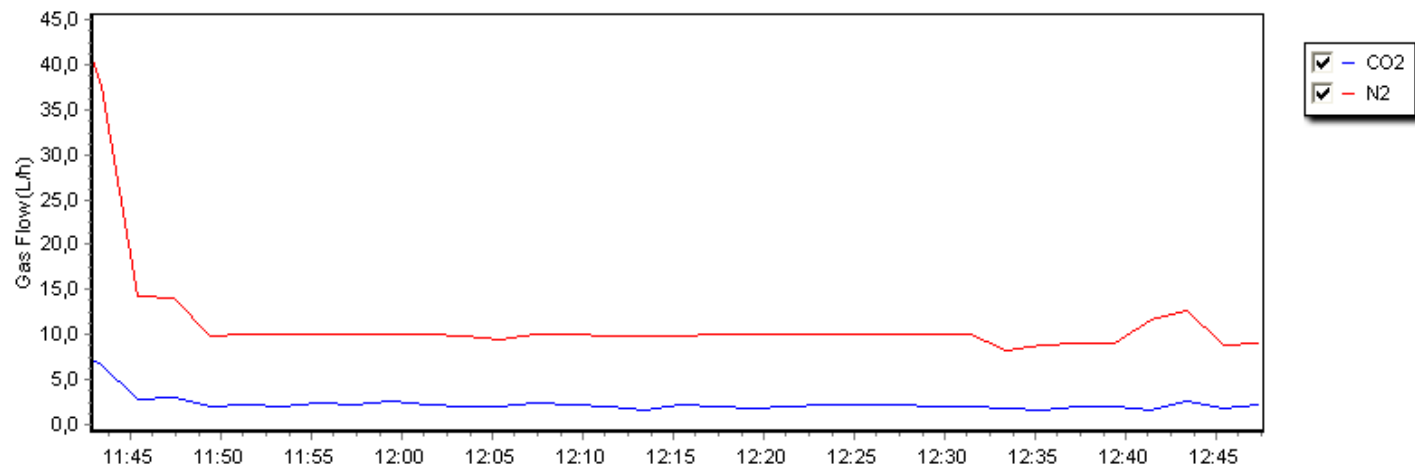
Technical validation: pre production model

- User interface software: bugs removed – extra functionality added
- Minor changes in design: improvements on seals – design of gas mixing chamber
- Requirements on safety
 - Development of monitoring software
 - permanent monitoring and registration of critical parameters (Temp – CO₂ – O₂)
 - Audit trail
 - E-mail messages
 - Alarm switch connected to modem
 - Independent monitoring
 - Installation of Vaisala CO2 sensor in gas mixing chamber
 - Temperature monitoring?

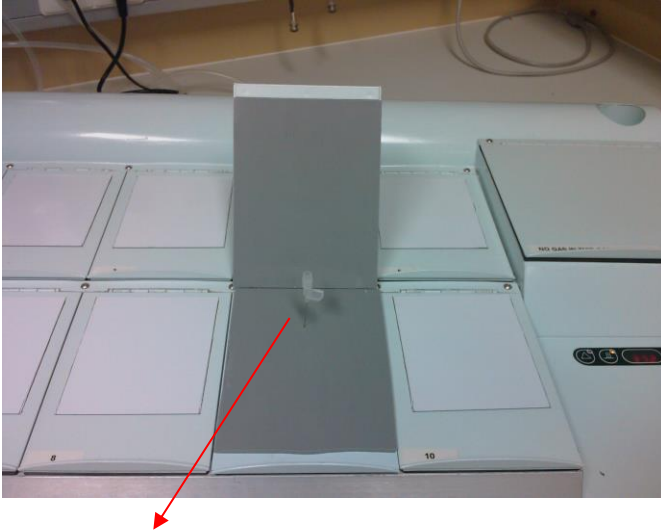
Monitoring software



Temperature and Gas Level **Gas Flow and Pressure** Daily Average Warnings Mail



Measurements



- Gas sampling in closed chamber
- Temperature: calibrated thermometer with thermocouple fixed in culture dish
- pH: ISFET probes – suitable for small volumes

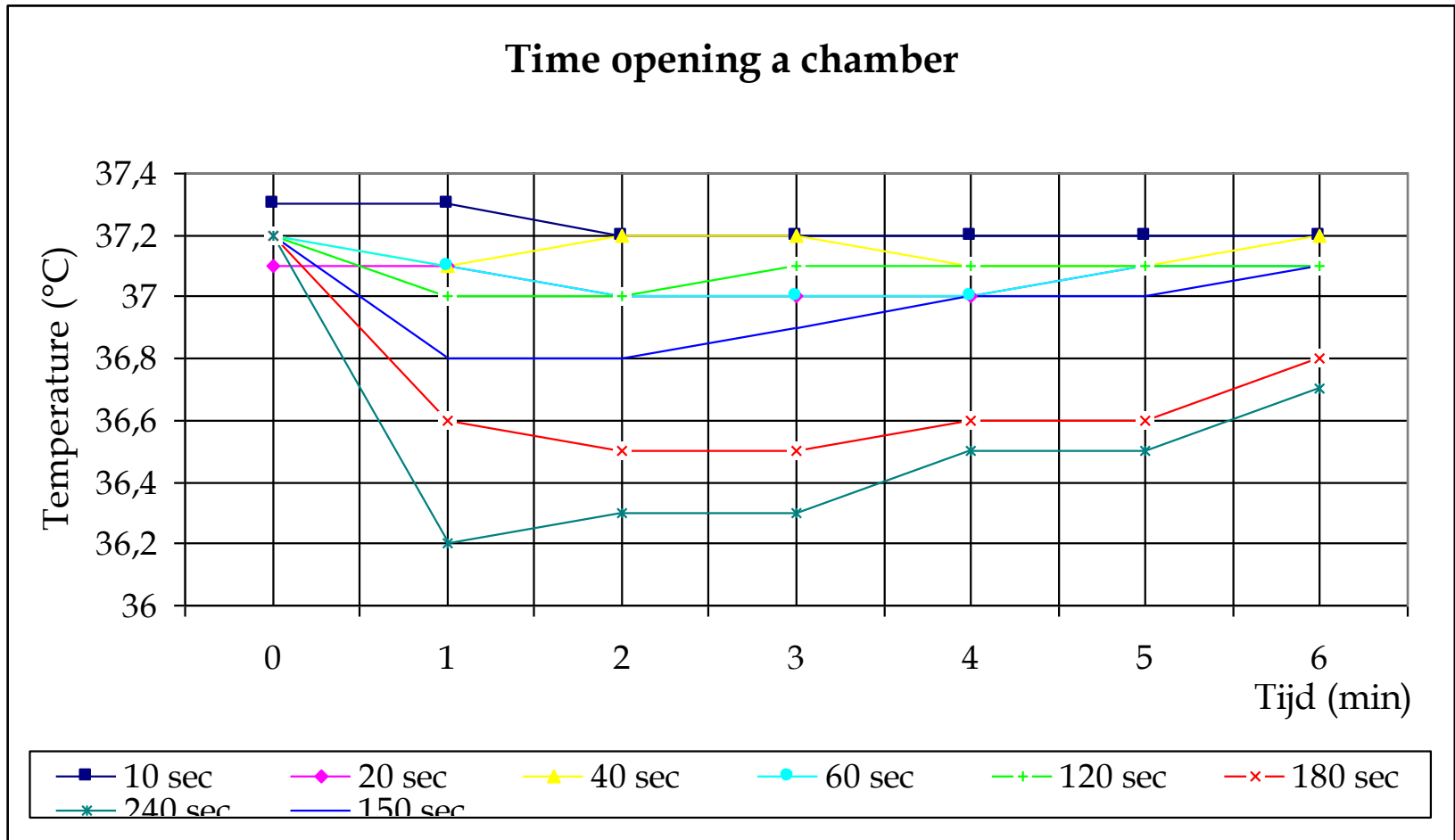
RI pH meter



Temperature – closed chambers

	Gemiddelde (°C)	Standaarddeviatie
kamer 1	37,18	0,04
kamer 2	37,26	0,05
kamer 3	37,18	0,10
kamer 4	37,18	0,04
kamer 5	37,19	0,07
kamer 6	37,21	0,05
kamer 7	37,17	0,05
kamer 8	37,24	0,05
kamer 9	37,14	0,08
kamer 10	37,21	0,05
Gemiddelde waarde	37,19	0,05
	<u>t-test</u>	
<u>p-waarde</u>	0,217	

Temperature in Nunc 4well dish in opened chamber

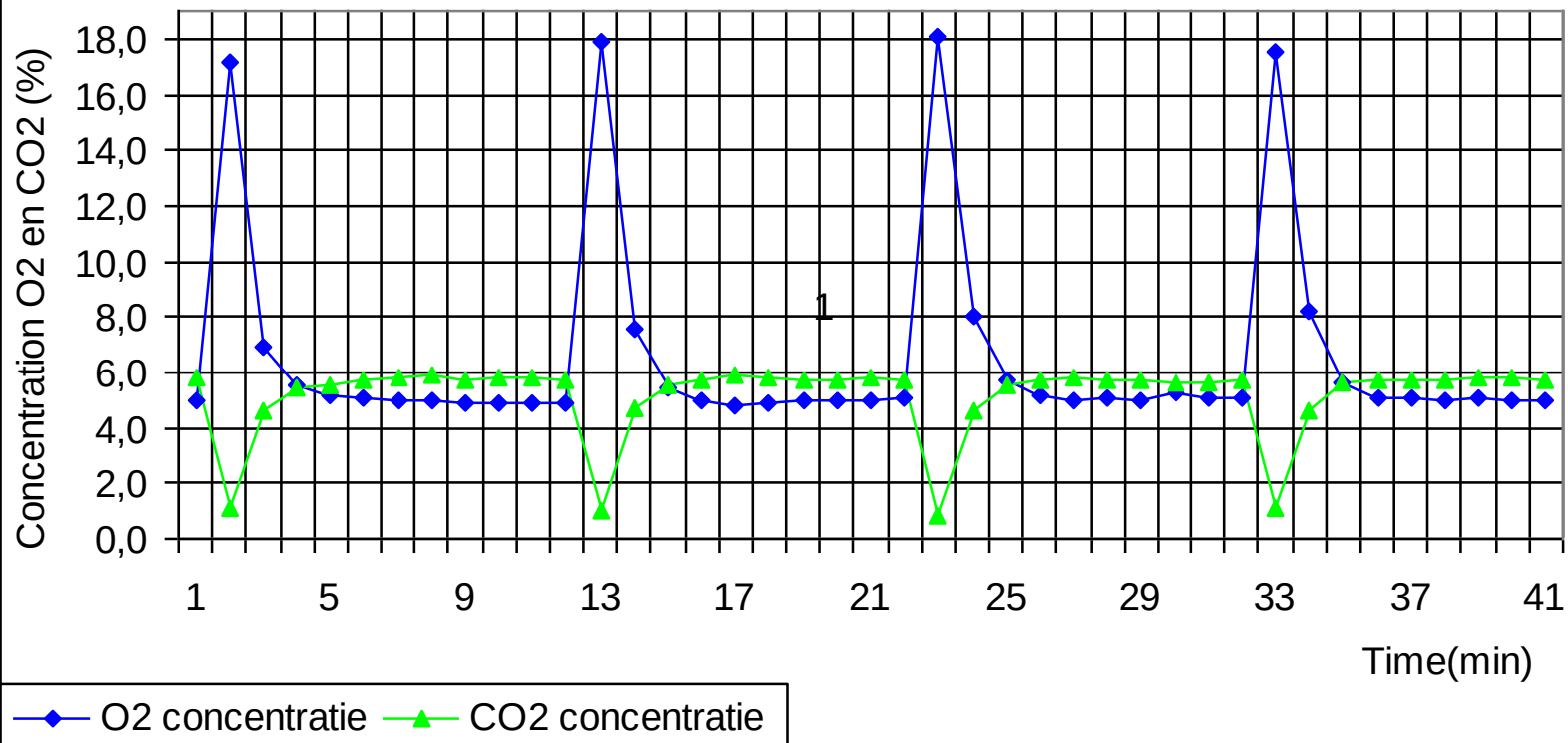


Gas concentration (closed chambers)

	Average (n=10)		Stdev	
	[O ₂]	[CO ₂]	[O ₂]	[CO ₂]
Chamber1	4,9	5,9	0,16	0,09
Chamber2	4,8	5,9	0,11	0,11
Chamber3	4,8	5,8	0,12	0,10
Chamber 4	4,9	5,9	0,14	0,13
Chamber 5	4,7	5,9	0,08	0,16
Chamber 6	4,8	6,0	0,13	0,13
Chamber 7	4,7	5,9	0,04	0,05
Chamber 8	4,8	5,9	0,04	0,09
Chamber 9	4,9	5,9	0,07	0,06
Chamber10	4,9	5,9	0,11	0,15
Average	4,8	5,9	0,10	0,11
t-test				
	O ₂		CO ₂	
p-waarde	0,00		0,168	

Recovery time

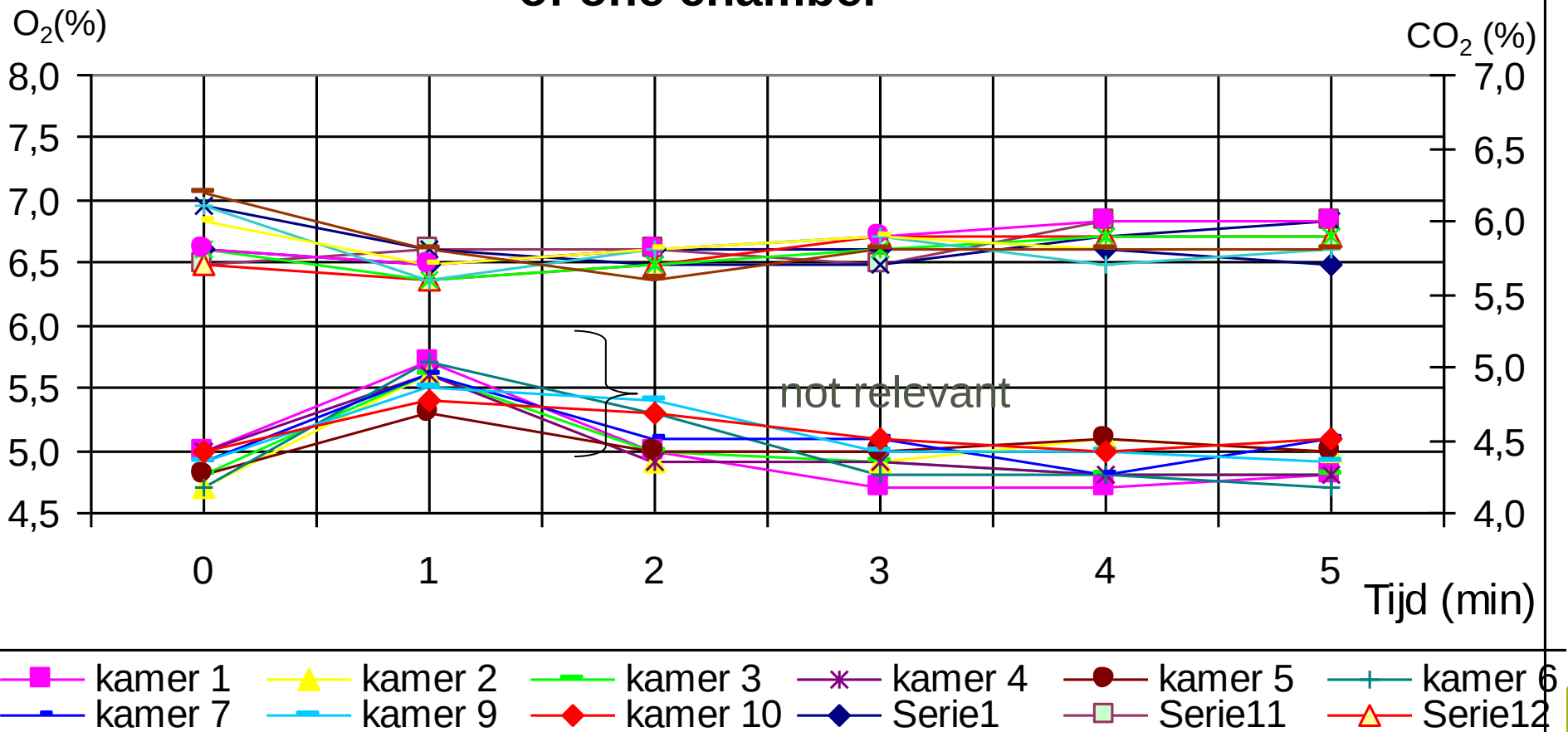
CO₂ en O₂ after opening one chamber



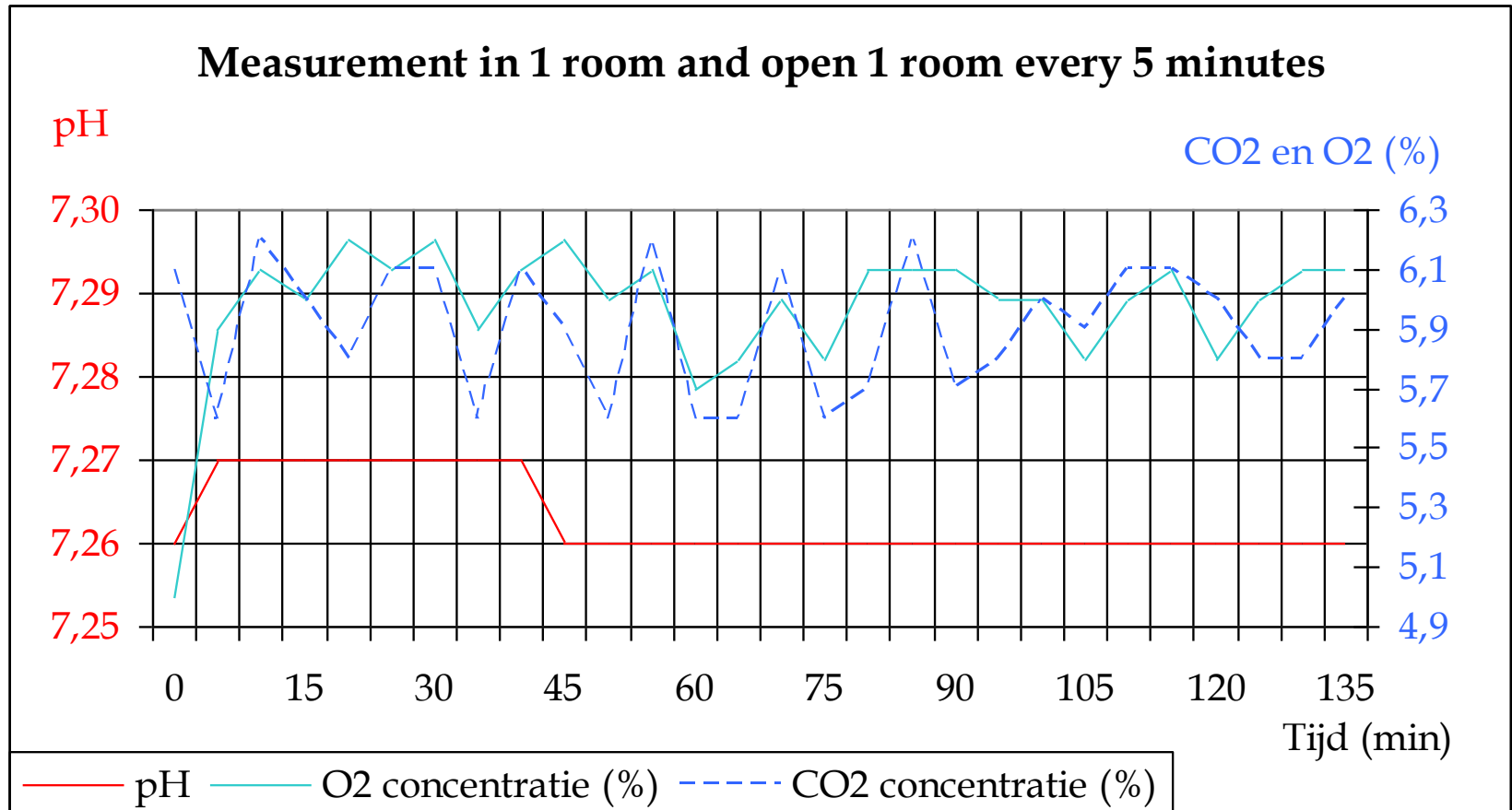
Fast return to homeostatic conditions: 3 minutes

Impact of opening one chamber on gas concentrations

O₂ en CO₂ in closed chambers after opening of one chamber



Impact of frequent opening



Particle count

	G185			10 incubatoren		
partikelgrootte	0,3 μm	0,5 μm	5,0 μm	0,3 μm	0,5 μm	5,0 μm
gemiddelde	17 380	472	0	205 700	24 510	192

Maximum permitted number of particles per m^3 equal to or greater than the tabulated size				
	At rest		In operation	
Grade	0.5 μm	5.0 μm	0.5 μm	5.0 μm
A	3520	20	3520	20
B	3520	29	352000	2900
C	352000	2900	3520000	29000
D	3520000	29000	Not defined	Not defined

Volume 4 - EU Guidelines for Good Manufacturing Practice Medicinal Products for Human and Veterinary Use – annex 1 (feb 2008)

Validation G185 Direct Heat Incubator

- * [O₂] **4,8%**
- * [CO₂]..... **5,9%**
- * Temperatuur..... **37,1°C**
- * pH **7,28**
- * Class A (GMP)

Requirements

5,0% ± 1%

6,0% ± 0,5%

37,0°C ± 0,5°C

± 7,20 – 7,30



Technical validation: conclusions

Closed incubator: homogeneous and stable gas and temperature distribution

After opening:

1. Fast recovery of gas conditions in opened chamber
2. No significant impact on other chambers

Conclusions

Optimal culture parameters are maintained

Chambers can be considered as individual culture

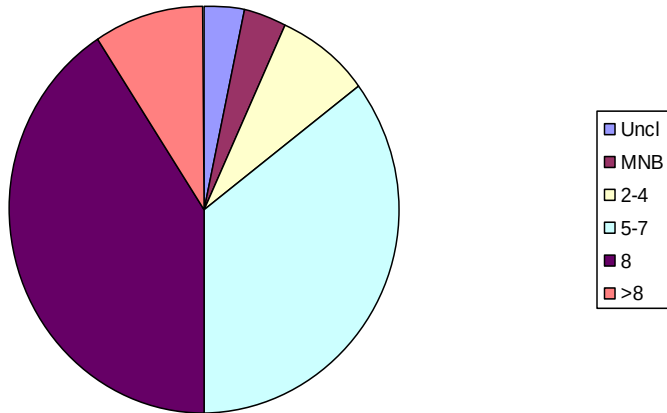
- Technical validation
 - Preproduction model
 - Final product
- Clinical validation
 - 3PN zygotes (n=20)
 - Sibling oocyte study: G-185 vs Heracell240
 - Randomised Controlled Trial (started 26/05/2009)

Sibling oocyte study (n=24)

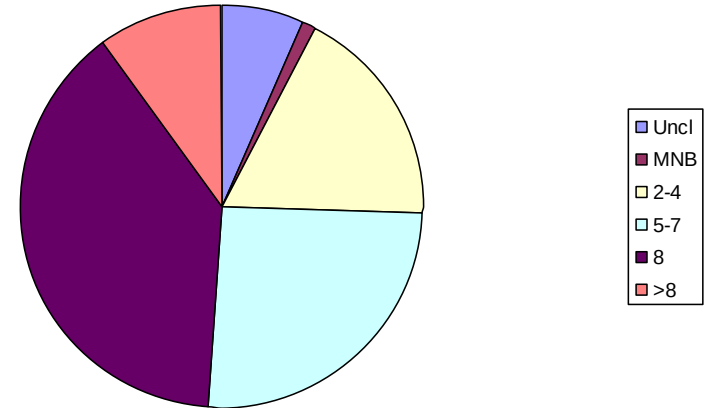
	G-185 N (%)	Heracell 240 N (%)	
Injected (inseminated)	126	133	
Deg	11	10	
1PN	9	2	
2PN	86 (68.3)	90 (67.7)	NS
3PN	3	2	

Embryo development day 3

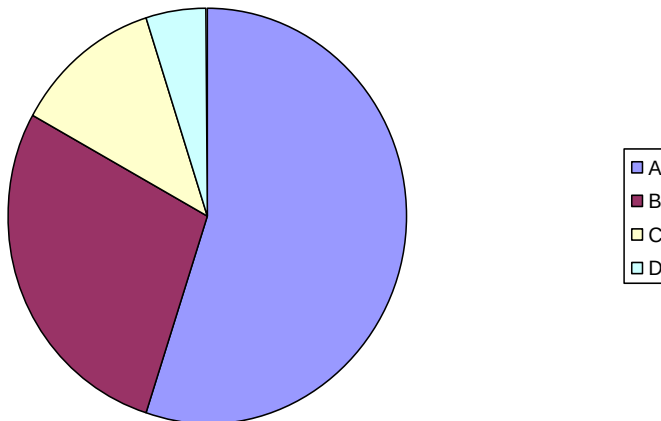
Cleavage stage D3 G-185



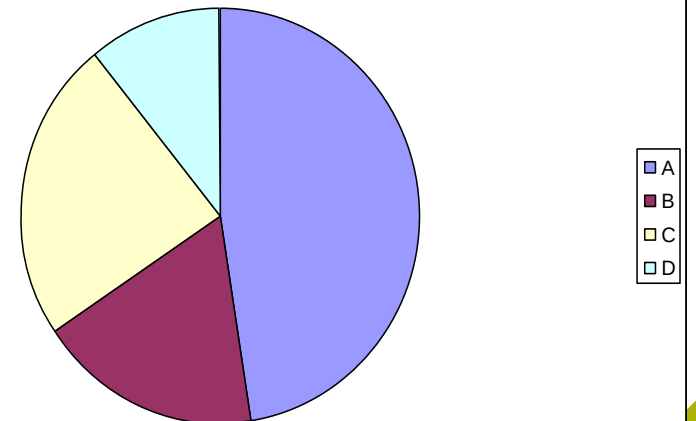
Cleavage stage D3 Standard



Embryo quality D3 G-185

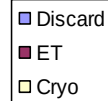
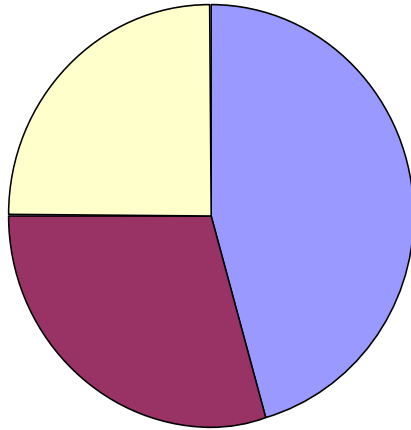


Embryo quality D3 Standard

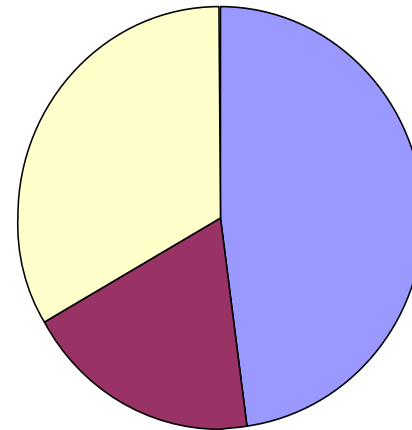


Embryo replacements - cyropreservation

G-185



Standard



Cycles	24	+hCG
No replacement	2	
Standard	4	1
Mixed	10	5
G-185	8	2



RCT

- Started: 27/05/2009
- Cycles randomised: 100 (26/06/2009)
- + hCG: ?

Conclusions

- Design specifications:
 - $37^{\circ}\text{C} \pm 0,5^{\circ}\text{C}$
 - $6\% \text{ CO}_2 \pm 0,5\% - 5\% \text{ O}_2 \pm 1\%$
 - Stable culture conditions
 - ...
- Functional specifications:
 - Grow viable embryos
 - Good embryo quality
 - Easy to use
 - Alarm functions
- User requirements:
 - Use as safe incubator for IVF
 - Space saving alternative for standard incubators

