



Janet Padgham, Director  
0418 204 910  
jpadgham@gytech.com.au



Susie Bilson, Account Manager TAS  
0418 204 991  
sbilson@gytech.com.au



Bree Plunkett, Account Manager NSW  
0418 204 476  
bplunkett@gytech.com.au



Julie Isbill, Account Manager NSW  
0438 363 452  
jisbill@gytech.com.au



Ross Turner, Account Manager NSW  
0418 204 775  
rturner@gytech.com.au



Kerrin Ball, Account Manager VIC, SA, WA  
0418 204 904  
kball@gytech.com.au

## Near-infrared (NIR) analysis of Day 2 Single Embryo Transfer Culture Media Samples: Blind Validation of Multi-linear Regression Algorithm

L. Botros<sup>1</sup>, H. Morita<sup>2</sup>, O. Kato<sup>2</sup>, N. Yamashita<sup>2</sup>, D. E. Seli<sup>3</sup>, D Sakkas<sup>1,3</sup>.

**Objective:** Non-invasive metabolomic profiling of embryo culture media using NIR spectroscopy correlates with the reproductive potential of embryos. This technology assesses modifications of the chemical composition of the embryos surrounding medium using spectroscopy and generates a value termed the ViaMetrics-ETM score. In the current study our objectives were to: (i) develop a model for the calculation of the ViaMetrics-E score for embryos transferred on day 2 and (ii) determine whether a blinded analysis of day 2 embryo culture media using this model could predict their reproductive potential.

**Design:** NIR analysis of Day 2 media samples and correlation with fetal cardiac activity (FCA).

**Materials and Methods:** SETs (n=181) were performed on Day 2 at the Kato Ladies Clinic, Tokyo, Japan. Day 2 embryos were assessed by morphology and the best single embryo transferred. After SET the embryo media and a blank were cryostored. NIR spectral analysis was performed based on technology involving two steps: 1. Computation of a mathematical formula (multi-linear regression) that correlates key NIR spectral regions to an embryos metabolic viability. The methodology serves to identify spectral differences between transferred embryos which did (+) and did not (-) result in FCA. A genetic algorithm determines these spectral regions, which are expressed as independent variables in a multi-linear regression. A dependent variable, ViaMetrics-E

score is calculated from the resulting regression. 2. Blind validation of the multi-linear regression algorithm on the NIR spectra of an independent set of Day 2 culture media.

**Results:** NIR spectroscopic analysis of spent culture media of embryos with proven reproductive potential demonstrated higher ViaMetrics-E scores (mean + SD) in both studies.

*ViaMetrics-E scores of pregnant & non-pregnant patients*

Study	N	ViaMetrics-E Score FCA +	ViaMetrics-E Score FCA -	P value (T-test)
Model Development	121	0.34 ±0.17	0.24 ±0.16	0.002
Blinded Assessment	60	0.33 ±0.18	0.26 ±0.15	0.048

In the blinded assessment the algorithm had an accuracy of 72% in identifying viable and non-viable embryos. When the 60 blinded samples were separated into quartiles, of increasing ViaMetrics-E scores, there was a positive correlation between ViaMetrics-E scores and positive FCA (Pearsons; P<0.05).

**Conclusions:** Findings of the current study suggest that the ViaMetrics-E score can provide an independent parameter that may significantly improve embryo assessment.

# Non-Invasive Metabolic Profiling of Day 5 Embryo Culture Media adds to the Discriminatory Power of Blastocyst Culture for Single Embryo Transfer

T. Hardarson, M. Tucker, E. Seli<sup>1</sup>, L. Botros<sup>4</sup>, P. Roos<sup>4</sup>, D. Sakkas<sup>1</sup>.

**Objective:** We have recently reported that non-invasive metabolomic profiling of embryo culture media using near infrared (NIR) spectroscopy correlates with the reproductive potential of embryos in IVF (Seli et al., 2007). This technology assesses modifications of the chemical composition of the embryos surrounding medium using spectroscopy and generates a value termed the ViaMetrics-E score. In the current study, we hypothesized that the use of a ViaMetrics-E score could further assist in determining the most viable embryo in addition to extended culture to blastocyst.

**Design:** NIR analysis of Day 5 embryo culture media samples and correlation with pregnancy outcome.

**Materials and Methods:** Single embryo Day 5 transfers (n=137) were performed at the Fertilitetscentrum, Gothenburg, Sweden and Shady Grove Fertility RSC, Rockville, Maryland, USA. Day 5 embryos were assessed for SET based on morphology. After SET a media sample from the transferred embryo and a blank were collected and cryostored. Media NIR analysis and a genetic algorithm were used to identify spectral regions, for eg. -SH, C=C, -CH, -OH, and NH groups, that discriminated most between transferred embryos that did and did not result in pregnancy (fetal cardiac activity detected at 12 weeks of gestation). Relative ViaMetrics-E scores were calculated by quantification of these spectral regions, adjusting for parallel blank media controls.

**Results:** The overall pregnancy rate from the two clinical sites using SET was 69/137 (50.4%). When the transferred embryos ViaMetrics-E scores were assessed, we found a large variability in metabolic profiles (range: -

A significant (Pearsons:  $P < 0.001$ ) positive correlation was observed with increasing ViaMetrics-E score quintiles and their associated implantation rates (Table 1).

*ViaMetrics-E scores of day 5 embryos & implantation rates*

ViaMetrics-E Score	Pregnant	Not Pregnant	Implantation Rate (%)
<0.2522	7	19	26.9
0.2522-0.4451	6	21	22.2
0.4451-0.5415	13	14	48.1
0.5415-0.7004	21	6	77.8
>0.7004	22	8	73.3

**Conclusions:** The ViaMetrics-E score helps discriminate between day 5 embryos, confirming that the embryos metabolism varies widely regardless of morphology. The added armory of metabolomic profiling by NIR spectroscopy, coupled with bioinformatics, to current morphological assessment protocols seems to allow a greater discrimination for selection of embryos for transfer, and has considerable promise to improve IVF outcomes.

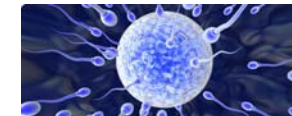
**Support:** This study was partly supported by Molecular Biometrics LLC.

For all your micropipette needs visit [www.humagenivf.com](http://www.humagenivf.com)

Gytech, Australian distributor for Humagen



## Sperm Analysis Slides



Leja slides are high quality disposable counting chambers. These slides were especially developed for semen analysis. Other uses have been found in many different fields like haematology and microbiology for example.

### Non-toxic.

The Leja slide is made using non-toxic glue, ink and coating. Samples therefore remain viable and can be counted for up to 45 minutes after loading the chamber.

### No bubbles.

Due to the consistently accurate chamber depth and specially developed coating, no air bubbles will occur when the Leja slide is loaded with a sperm sample.

### Less diluting.

Leja slides have a specially designed air vent that allows even viscose samples to spread evenly throughout the whole chamber.

## PURECEPTION SPERM WASHING

An 80% (v/v) or 40% (v/v) sterile colloidal suspension of silica particles stabilized with covalently bound hydrophilic silane formulated in HEPES-buffered human tubal fluid. The kit components will allow for the highly efficient separation of motile sperm from the ejaculate of most semen specimens.

Sterile filtered to remove particulates, improve stability, enhance performance and avoid sedimentation. Tested using one-mouse embryo bioassay and endotoxin assays to ensure quality and safety.

SART-2040: PureCeption 40% Upper Phase  
 SART-2080: PureCeption 80% Lower Phase  
 SART-2100: PureCeption 100% Isotonic Solution

## Christmas/2009 Trading

Gytech will close for 2008 at 5pm on Tuesday 23<sup>rd</sup> December and resume normal trading at 8.30am on Monday 5th January 2009.

Gytech would like to wish all of our loyal customers a happy holiday and safe and successful 2009.

## MTG Mini Straws

**MTG Mini Straws for Cryopreservation**  
**Gamma irradiated straws of various size**

- 0.25 cc, 133 mm with identification stick
- 0.25 cc, 90 mm with identification stick
- 0.25 cc, 133 mm with glass sealing balls
- 0.25 cc, 90 mm with glass sealing balls
- 0.5 cc, 133 mm with metal sealing balls

*Order your cryopreservation straws today through Gytech*