# A Multi-Center Evaluation of a Novel IVF Cryostorage Device in an Active Clinical Setting

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## Abstract

# Purpose

To evaluate the safety, function, and integration of a novel automated software-guided cryostorage system in an active IVF laboratory setting.

# Methods

The Investigational Device (ID) was installed at 3 IVF laboratories (sites: α, β, and γ). A total of 15 embryologists were trained to use the ID. Mock patient specimens containing mirrored live patient data were handled using the ID. Temperature readings were recorded every minute. Successful identification, storage, and retrieval of patient specimens by the ID were evaluated. To assess an $LN_2$ pressure builder, the frequency of use and events of workflow interruption were logged. Student's t-test was used to determine statistical significance.

# Results

The ID was in active use for 164 days total. During this time, 329 mock patient egg and embryo cohorts were handled by the ID. The mean ± SD temperatures during active use were: α, -176.57 ± 1.83$^{O}$C; β, -178.21 ± 2.75$^{O}$C; γ, -178.98 ± 1.74 and did not differ significantly. The highest recorded temperatures were: α, -165.14$^{O}$C; β, -157.41$^{O}$C; γ, -164.45$^{O}$C. A total of 1064 automation transactions on 409 specimen vessels were performed. Data was managed on 1501 eggs and embryos. The ID did not lose or misplace any specimen data or vessels, and no specimen was exposed to a detrimental (>-150$^{O}$C) temperature excursion. Over the 25 $LN_2$ pressure builder usages during 99 total days, there was 1 occurrence where usage interrupted workflow due to a lack of $LN_2$ pressure.

# Conclusions

The ID has advantages over the current manual-based cryostorage systems, including radio frequency identification (RFID) tracking, automation of manual tasks, and software guidance to ensure accurate specimen storage and retrieval. The results of this study indicate that the ID can be easily integrated into active IVF laboratories.


## Introduction

There is increasing demand for the cryostorage of human reproductive specimens [1–12]. Human reproductive specimen cryostorage handling remains intensely manual [13]. Specimens are manually retrieved from storage with variability in handling [14]. Specimen identification and location commonly

rely on written or transcribed records and labels without the benefit of advanced software identification and tracking [14].

Routine cryostorage equipment includes liquid Nitrogen ($LN_2$) Dewars that store specimens at a single level and occupy increasing laboratory space [1, 15]. Modern assisted reproductive technology (ART) facilities arduously monitor cryostorage equipment stability and integrity with minimal software oversight [13, 15]. Embryologists have reported signs of fatigue, stress, anxiety, and burnout under current laboratory operating conditions [13, 16–21]. Anxiety is known to be specifically associated with cryostorage working conditions [16–21], which may impact fidelity of the manual process. Embryologists working with manual cryostorage operations could benefit from the adoption of automation and software assistance within the laboratory setting [17–19].

Cryopreserved specimen mis-labeling errors, although rare, are known to occur [22]. The variability in specimen identification and handling, along with the reliance on non-digital identification and data handling, is likely to contribute to these rare occurrences of specimen mix-up and error [13]. It is believed that this similarly contributes to the fatigue, stress, anxiety, and burnout experienced by embryologists in cryostorage working conditions.

The present study evaluates a novel IVF specimen cryostorage system (Investigational Device – ID). The study objective is to evaluate the safety, function, and integration of the ID in an active clinical setting. The ID includes automation intended to support embryologists and their working conditions by eliminating or reducing many manual tasks and facilitating specimen identification into, during, and from storage. The ID couples automation with specifically designed software to (1) provide active oversight of environmental conditions, (2) ensure proper equipment function, (3) enable an auditable digital chain of custody, and (4) lessen variability of specimen identification and retrieval from storage.

## Materials and Methods

# Investigational Device

The ID (Fig. 1) consists of a cryostorage tank, temperature and environmental sensors, RFID readers, automation, an $LN_2$ pressure builder (Apollo®, Cryotherm GmbH & Co. KG, Kirchen (Sieg), Germany), and software. The cryostorage tank is a vacuum-insulated 250L liquid nitrogen storage vessel. The tank stores up to 1383 CryoBeacons (Fig. 2) in 2 levels of racks suspended in the vapor phase of $LN_2$. CryoBeacons are specimen receptacles designed to hold common commercially available reproductive health cryodevices with vitrified reproductive specimens. CryoBeacons are maintained below − 150$^{O}$C during storage through the cooling effect of the $LN_2$ vapor.

The CryoBeacons are RFID-tagged receptacles. The ID has multiple RFID antennae to identify CryoBeacons, determine location, and distinguish the desired CryoBeacon from others in close proximity.

All CryoBeacons are identified via the RFID tag at least twice during specimen deposit and withdrawal from the ID.

CryoBeacons are submerged in $LN_2$ in specifically designed carriers that are placed into the ID by trained embryologist operators. The embryologist interfaces with the ID through an iris scanner and a touchscreen control. Once placed into the ID automation moves the desired CryoBeacon from the $LN_2$ carrier to the storage location in the tank. Specimen cryogenic temperature is maintained during movement by residual $LN_2$ in the CryoBeacon during movement. The ID confirms that the CryoBeacon has sufficient $LN_2$ to maintain specimen thermal integrity before movement. Should automation fail during movement, an emergency $LN_2$ feed line floods the CryoBeacon. The emergency $LN_2$ feed requires 35 psi supply. The ID will not operate if 35 psi is not measured. To ensure 35 psi, the $LN_2$ pressure builder is part of the ID and evaluated in this study.

The ID is controlled by custom-designed software including ivfOS™. The software functions to control access to registered users, ensure correct placement and location of CryoBeacons, control automation, read and log data from temperature and environmental sensors, enable offsite monitoring of safety and operations, and maintain an auditable digital chain of custody of specimens.

# Study Sites and Study Conduct

The ID was installed at three study sites (α, β, and γ). All three sites had experience with automated cryostorage equipment, but not the ID. A total of 15 embryologists (4 at α and γ, 7 at β) were trained to use the ID and participated in the study.

To evaluate the integration, function, and safety of the ID in an active IVF laboratory, mock patient freeze cohorts consisting of blank CryoBeacons (CryoBeacons without cryodevices) were registered with the software and deposited into the ID for storage. No live specimens were used in this evaluation. Live patient specimen data was mirrored in ivfOS™. The patient's live specimens and data were handled throughout the study by the site using their individual manual processes and procedures. Five patient cohorts (all eggs or embryos from a single oocyte retrieval even if there were multiple days of freezing) were deposited per day (exclusive of weekends) throughout the 30-day study period. One hundred patient cohorts per study site were targeted. Following the first week of the study, on each weekday, 1 patient specimen from a cohort deposited a week earlier was retrieved and thawed (reported as thawed in ivfOS™). Similarly, if any patient's live specimen(s) were thawed the blank CryoBeacon was retrieved from the ID and the specimen(s) were reported as thawed. This ensured that the retrieval and return of CryoBeacons for further storage of remaining specimens was evaluated. To evaluate specimen safety during storage, temperature readings from a resistance temperature detector (RTD) near the specimen storage level in the tank were recorded every minute.

The software allows for the printing of cryodevice labels. The cryodevice labels are linked to the patient's data record and the assigned CryoBeacon(s). Since there were no cryodevices used in the evaluation, the labels were adhered to a paper data record for each patient cohort. At the conclusion of the study, any

remaining CryoBeacons were retrieved from the ID. The physical CryoBeacons were then matched to the inventory for the ID in ivfOS™ and the physical labels on the paper data records. Any discrepancy between the three databases was investigated for the root cause to determine if the fault was due to the ID or human error.

To evaluate the integration of the $LN_2$ pressure builder into a busy IVF laboratory, the frequency of use and workflow interruptions were logged. Included in the log were questions if the use was expected or unexpected, if it disrupted work or was planned, and if the embryologist was able to complete the planned task they were performing.

# Statistical Analysis and Ethics

Student's t-test and descriptive statistics were used to evaluate temperatures. Counts of misplaced, misidentified, or lost specimen receptacles were used to evaluate the digital chain of custody.

The study protocol was reviewed and approved to be exempt from IRB oversight (Pro00067860, Center for IRB Intelligence (CIRBI) Platform, Advarra, Columbia, MD).

# Results

The ID was in active use for 164 days total (36, 68, 30; α, β, γ, respectively). During this time, 329 mock patient egg and embryo cohorts (56, 173, 100) were handled by the ID. Site β conducted a preliminary study using the same protocol, without the $LN_2$ pressure builder, prior to initiation of sites α and γ. Site α is a satellite laboratory with smaller patient volume than sites β and γ.

The mean ± SD temperatures during active use were: α, -176.57 ± 1.83$^{O}$C; β, -178.21 ± 2.75$^{O}$C; γ, -178.98 ± 1.74 and did not differ significantly. The highest recorded temperatures were: α, -165.14$^{O}$C; β, -157.41$^{O}$C; γ, -164.45$^{O}$C (Table 1).

Table 1
No specimens tracked by the device were exposed to a detrimental temperature excursion (> -150°C) throughout the 164 combined days of active use.

| Site | α | β | Γ |
|---|---|---|---|
| Mean ± SD temperatures during active use | -176.6 ± 1.8°C | -178.2 ± 2.8°C | -178.9 ± 1.7°C |
| Highest recorded temperatures | -165.1°C | -157.4°C | -164.5°C |

A total of 1064 automation transactions on 409 specimen vessels were performed. Data was managed on 1501 eggs and embryos. The ID did not lose or misplace any specimen data or vessels, and no specimen was exposed to a detrimental (>-150$^{O}$C) temperature excursion (Table 2).

Table 2
No specimens or specimen data tracked by the device were lost or misplaced; A total of 1064 automated transactions and 1501 specimen data were handled.

| Site | α | β | Γ |
|---|---|---|---|
| Mock Patient Cohorts | 56 | 173 | 100 |
| Lost or Misplaced Specimens | 0 | 0 | 0 |

The $LN_2$ pressure builder requires periodic filling (~ every 3 days, depending on use). Each site chose to use the $LN_2$ pressure builder with different determination factors, frequencies, and time of day. Site α used the pressure builder 10 times over 36 days, while β used the system 6 times over 33 days and γ 9 times over 30 days. Over the 25 $LN_2$ pressure builder usages during 99 total days, there was 1 occurrence where usage interrupted workflow due to a lack of $LN_2$ pressure.

# Discussion

There is an increasing demand for fertility services, including cryostorage of reproductive health specimens [1–12]. It is believed that this increasing workload is leading to high levels of stress, fatigue, burnout, and anxiety regarding cryostorage operations reported by embryologists [13, 16–21]. However, even with the increasing number of IVF cycles compounding issues, cryostorage operations still commonly rely on handwritten labels and paper ledgers to track, locate, identify, deposit, and retrieve specimens, in addition to manual regulation of cryostorage equipment function and environmental conditions [1, 13–14]. The introduction and adoption of technological improvements, including dedicated software management, is lagging in IVF cryostorage operations [15].

Embryologists working in IVF cryostorage facilities spend an inordinate amount of time doing fatiguing work [13]. It is believed this is a result of the current cryostorage equipment design and lack of automation and software. Embryologists in the UK and US report a desire for technological improvements in cryostorage operations [17–19].

This report is the evaluation of the safety, function, and integration of an ID for reproductive health specimen cryostorage. The ID combines software with automation. These features function together to provide a robust digital chain of custody with oversight of equipment function to help ensure specimen integrity and operator safety.

The ID functioned as intended during the study period. The paramount concern with specimen integrity is temperature excursions beyond the devitrification temperature that may harm or destroy them [23–25]. During the 164 days of use in an active clinical setting there was not a temperature excursion that would have placed a specimen in jeopardy.

To function correctly and provide for a digital chain of custody, the software and automation of the ID must work together. In this study, the ID did not lose, misplace, or misidentify any specimen receptacle. Even though the study sites chose to integrate the ID, especially the $LN_2$ pressure builder, in different ways, the data support that the $LN_2$ pressure builder does not disrupt the workflows of busy IVF laboratories.

In summary, the ID is easily integrated into IVF laboratories, functions as designed, and is safe for specimens and trained operators. There are benefits of the specific ergonomic design, that limit physical strain, such as the need to bend over, lift heavy objects, or stand on stools and ladders [13]. These design benefits, when coupled with the accurate specimen identification and location from the digital chain of custody, should improve embryologist working conditions and the reported levels of cryostorage-related anxiety [17–19]. Using software to monitor specimen and environmental conditions and labware function eliminates the need for written records and ledgers and allows for analytics to improve laboratory operations.

## Declarations

# Disclosures

Michael G. Collins Ph.D., Robert Woodhull, Ellen Stringfellow and Ashley Souza are all fulltime employees of TRMW Life Sciences.

# Author Contribution

M.C. designed the experiment, oversaw data collection and analysis, wrote the main manuscript text. All authors contributed to the concept of the study. All authors reviewed and approved the manuscript. J.B., J.T., N.L., C.M., A.D., J.M., A.L., S.P., E.K., R.S., and L.R. conducted the experiment. R.W., E.S. and A.S. provided logistical support for equipment, training on appropriate use and oversight during data collection.

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# Figures


**Figure 1**

Investigational Device: a novel automated software-guided cryostorage system.

## Figure 2

CryoBeacons: RFID tagged vessels submersed in $LN_2$ for cryopreservation and storage.of specimens.