

A Protocol for Implementation and Use of A Tissue Incubator for Rapid Corneal Warming at the Eye Bank

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ABSTRACT

Purpose: To share a protocol and the experience of implementing a tissue incubator and rapid warming procedure to increase the quality and efficiency of corneal evaluations at an eye bank.

Method: A method is described for selecting a suitable tissue incubator, equipment qualification process (installation qualification, operational qualification, and performance qualification (IQOQPQ)), active temperature monitoring, and cleaning and maintenance schedules. Also shared is the incubation protocol prior to tissue evaluation for newly recovered donor corneas and the documentation procedures. Finally, included is a discussion of key considerations for changes to standard operating procedures as well as the benefits of improved work flow at the eye bank.

Results: The qualification process for this incubator was performed over a 3.5-week period (from design through approval). Temperature tracking during the testing phase showed that the equipment worked properly and maintained the desired temperature range. Incubation protocols were established and implemented with minimal difficulties.

Conclusion: Rapid warming has helped improve work flow at the eye bank and has allowed staff to reliably obtain superior specular images.

Key Words: Implementation of rapid tissue warming; Corneal tissue evaluation; Tissue evaluation time; Eye bank tissue warming; Tissue incubation.

The ability to obtain an accurate evaluation that properly reflects the condition of donor corneas is important for maximizing their transplantation potential, and provides surgeons with the crucial information needed

to make their final tissue suitability determination.¹⁻³ This process typically includes the acquisition of a specular image for analysis of endothelial cell counts and morphometric parameters, and a slit-lamp evaluation of the whole cornea to identify tissue damage or defects that may affect the performance of the donor cornea after transplantation.

The process of obtaining a specular image can be time consuming because technicians must wait for corneas that are removed from cold storage and placed at room-temperature to sufficiently warm prior to image acquisition. Because corneas warm-up at different rates when warmed at room-temperature,^{4,5} technicians must check the corneas frequently to capture a specular image, complete the evaluation process, and return the donor corneas to cold storage in order to minimize tissue exposure time to elevated temperature outside of the recommended storage temperature range.³

Recently, our eye bank has validated a protocol to warm donor corneas to near physiological temperatures (35°C) in a tissue incubator for up to 2 hours to achieve better control of the tissue warming step.⁵ We have further quantified and showed that this protocol, once implemented at the eye bank, allowed technicians to dramatically reduce overall tissue evaluation time and in turn also reduce the time corneas are exposed to elevated temperatures outside of the recommended tissue storage conditions.⁶ Thus, rapid corneal warming has helped improve eye bank workflow efficiency for tissue evaluation and allowed for reduced tissue exposure to conditions that may promote harmful pathogen growth.

This report describes the experience of selecting and qualifying appropriate equipment, implementing a rapid

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warming protocol, and shares an example protocol in detail. The report also discusses the potential for a standardized method of documenting and reporting tissue warming times so that importing eye banks can determine whether import corneas can safely be exposed to additional warming cycles when necessary.

METHODS AND RATIONALE

Implementation Protocol

Choosing the 'right' incubator

Several steps were taken to reduce the possibility of over-incubating (i.e. over-heating) donor corneas. In choosing an appropriate incubator, two important features that were high on the priority list were 1) the overall size, and 2) the incubator should have a clear door or window. The incubator needed to be large enough to incubate up to 12 corneas at a time for work flow purposes, while small enough to fit neatly, but visibly, on the counter. The clear door or window would allow staff to see the inside of the incubator without opening the incubator door. This allows staff to visualize the contents within the incubator while keeping the internal temperatures as stable as possible.

Another important requirement that can easily be overlooked is the operating temperature range of a given incubator. A previous laboratory study showed that it was safe and effective to warm donor corneas at near-physiological temperatures of $\sim 35^{\circ}\text{C}$,⁵ while other work has shown that corneas can safely be exposed to $\sim 37^{\circ}\text{C}$,⁷ which is well within the standard temperature range of most incubators. Therefore, in selecting the 'right' incubator, be sure to quickly check the specifications to be certain that the incubator will work for your specific application.

One specification that was overlooked in the initial selection process is the ability to actively and continuously monitor the internal temperatures within the incubator. If you wish to implement an automatic and active environmental monitoring service, look for an incubator with a temperature monitor port. The incubator below does not have a specified port, but a probe can still be run into the incubator without adverse effects (Fig. 1A).

Incubator Used:

- Heratherm™ Compact Microbiological Incubator (Fig. 1A)
- Model: IMC 18
- Manufacture: Thermo Fisher Scientific
- Operational temperature range: 17° - 40°C



Figure 1A Heratherm™ Compact Microbiological Incubator. Two corneas inside of their viewing chambers can be seen through the clear door. The orange arrowheads show where the temperature probe for active monitoring is located inside of the incubator (bottom arrowhead) and where it exits the incubator.

The IQOQPQ

The device was qualified by following an Installation Qualification, Operation Qualification, and Performance Qualification (IQOQPQ) process. This is a quality control process that documents and ensures the equipment is installed per manufacturer's guidelines, operates as the manufacturer intended, and that it performs as intended by both the manufacturer and user. Each eye bank may have their own version of this process.

IQ: The Installation Qualification is the first step, and is a checklist taken directly from the Operational Manual (OM) of the incubator. This qualification step includes documenting the manufacturer's recommendations for installation, and then documenting that the device and its chosen environment meets each standard. This will vary depending on the manufacturer's recommendations. The OM should also be used to define minimum cleaning and maintenance protocols (see cleaning and maintenance section below).

At the end of each qualification step, note any deviations to the OM recommendations, list justifications for why these deviations do not impact operation, and explain any modifications implemented to compensate the deviation. For example, the Heratherm™ Compact Microbiological Incubator's OM recommends using a dedicated power outlet with a safety fuse (T16A) or B16 circuit breaker. We did not comply with this recommendation because this is a compact incubator that does not draw a lot of power. However, we were able to meet other outlet specifications, and we also utilized a surge protector. Thus, we felt comfortable with this deviation. We also did not have our incubator certified or calibrated by a third party during the initial installation because this was not part of the recommendations listed in the OM. Furthermore, we were able to complete our own testing and ongoing temperature monitoring to demonstrate temperature control.

OQ: The Operational Qualification is a three-step process that also follows the device OM. First, document and test

all described functions, e.g. the power button turns on the incubator, you can adjust the temperature, the temperature display shows numbers, etc. Next, identify all eye bank forms such as the standard operating procedures (SOP), work aids, and any other forms that will be affected by the implementation. Document completion of form updates for all applicable documents. Lastly, identify the personnel position descriptions that will interact with the incubator, and document the completion of necessary training, which may include creating new documents.

PQ: The last step in qualifying a new incubator is the Performance Qualification. This step documents that the device operates as the manufacturer describes, and that it meets your specific needs. For us, it was important that the incubator can warm corneas to near 35°C, without exceeding our safety threshold of 37°C. We examined this in two functional tests.

Test 1: Achieving and maintaining the desired temperature.

A previous series of laboratory studies validated that rapidly warming tissue with an incubator at 35°C produced the desired result of better quality specular images without negatively impacting cell viability or increasing pathogen growth compared to room-temperature warming.⁵ Testing for the new incubator was started by confirming that the incubator can maintain a constant internal temperature of ~35°C. The incubator was examined for a possible difference between the built-in temperature display, and the actual internal temperature. Using a Dickson data logger (SP125, Dickson, Addison, IL), it was found that the new incubator was consistently 2-degrees warmer internally than the display reflected (e.g. when the display was set to 35°C, the internal temperature was 37°C). Therefore, to achieve the desired internal temperature of 35°C (confirmed by the data logger), the incubator was set to 33°C. The SOP was written to reflect that the incubator be set at 33°C.

Subsequently, the internal temperature was tracked to ensure that it was maintained within $\pm 2^\circ\text{C}$ of the desired 35°C as measured by a data logger over an 8-hour period. The lower temperature limit (e.g. 33°C) is not important for safety reasons, but it is critical that the temperature inside of the incubator does not exceed 37°C.

Test 2: Confirming the desired heating effect.

A previous laboratory study showed that the Optisol-GS solution inside of a viewing chamber can be heated to ~35°C after 2 hours of incubation. Therefore, the second functional test was designed to ensure that this could be reproduced with the new incubator. To this end, 5 viewing

chambers filled with 20 mL of Optisol-GS were placed into cold storage for 48 hours. Following cold storage, all 5 chambers were moved to the incubator and incubated for 2 hours. After 2 hours, the temperature of the Optisol-GS was measured using a handheld digital temperature gauge (MA-Line, Arlington Heights, IL) and documented. The temperatures of the Optisol-GS should not exceed 37°C during this test period to maintain a sufficient safety buffer.

Cleaning & Maintenance

The incubator should be cleaned and maintained regularly by trained personnel. The OM will describe what and how the incubator should be cleaned, and you can determine on what schedule the cleaning should be performed. We implemented weekly and monthly cleaning, which is documented per our SOP. The cleaning and maintenance will vary depending on the manufacturer.

The cleaning protocol includes checking and emptying the condensation tray on a weekly basis (to date there has never been condensation in the tray); and monthly, disinfecting the interior and exterior surfaces. For the monthly maintenance, the following steps are followed:

Turn off and unplug the incubator prior to performing these steps.

Exterior surfaces should be wiped clean with Super Sani-Cloths (Professional Disposables International Inc., Orangeburg, NY) or similar product, then wipe dry with a paper towel.

For interior cleaning, be sure to first remove all samples and shelves. Wipe removed shelves and interior with disinfectant and dry with cloth. Ensure cleaning agent is completely removed before replacing shelves.

Additionally, the OM recommends annual inspection of the control panel, temperature controller, and electrical safety checks. We have opted not to implement this recommendation, because a nonfunctioning control panel will be obvious, and we are actively monitoring the internal temperature.

Temperature Tracking

While active temperature tracking was not a part of the original implementation process, we now use viewLinc (Vaisala Corp., Helsinki, Finland) environmental monitoring and reporting software with our incubator, which means the internal temperature is measured and recorded constantly. With this active tracking, if the temperature goes out of range, someone will be notified via text and/or email. We defined the upper temperature limit at 37°C, but did not specify a lower limit. This also provides a continuous log

of temperature ranges, which can be reviewed regularly to monitor adequate temperature regulation (Figure 1B). If the cost of active environmental monitoring is prohibitive, another option would be manually confirming the temperature and documenting the findings on a log, similar to how eye banks (used to) monitor transplant refrigerators.



Figure 1B Example of a temperature trace over a 2-week period.

Incubation Protocol

To regulate and maintain control of the incubation procedure and equipment, only trained eye bank staff are authorized to load, unload, clean and maintain the incubator. Training consisted of verifying the staff members' understanding of all work aid and SOP documents related to the incubator and tissue warming procedures. Below are highlighted areas that should be considered when designing and implementing an incubation protocol.

1. Volume (number of corneas warmed at one time).

It is important to avoid overloading the incubator. The maximum volume will vary, depending on the incubator size and how many corneas your team can evaluate at one time.

2. Positioning

- a. For maximum warming efficiency, viewing chambers should not be in contact with one another or the sides of the incubator. This allows for the warm air to circulate around all viewing chambers.
- b. Corneas should be placed near where the temperature measurements were taken during the qualification process. In our case, the shelves within the incubator are placed near the top of the incubator (on the top 2/3 of the incubator).

3. Warming time and temperature

- a. *Time:* Our previous laboratory validation showed that it is safe and effective to incubate tissue for up to 2 hours at 35°C.⁵ However, we set our standard protocol to allow staff to incubate donor corneas up to 1.5 hours. Setting our protocol at 1.5 hours gives us a 30-minute buffer as a safeguard and one additional step of mitigating the risk of accidentally overheating the tissue. A nonconformance is required for tissue incubated longer than 2 hours. In our experi-

ence, corneas can be ready for specular microscopy after 1 hour and 15 minutes of warming with superb results.

- b. *Temperature:* Based on what was learned during our Performance Qualification process, our incubator is set to 33°C to achieve internal temperature of 35°C. The incubator remains on at this setting at all times, except during regularly scheduled cleaning and maintenance. **We do not turn our incubator on and off daily, as this likely adds additional stress to the incubator motor.** Warming at 35°C provides effective results while providing a safety buffer to prevent corneas from being overheated.
- c. *Warming cycles:* Based on the previous validation,⁵ we allow two incubation cycles, but this is rarely used. One of the perks of using an incubator is that the tissue is predictably ready. This has increased evaluation efficiency and decreased the duplication of work (multiple people checking tissue readiness throughout the day).

4. Documentation

Documentation of the date and times in which chambers are both put in and pulled out of incubation is required per our SOP. Documentation is recorded on a tissue evaluation form unique to each tissue, and in our database. During incubation, these forms are kept close by the incubator, allowing staff to easily know which tissues are currently warming (Fig. 1C).



Figure 1C Tissue evaluation forms for corneas that are being warmed are kept close to the incubator during warming along with 1 of the 2 timers used to track warming times.

5. Timers

To ensure that corneas are removed from incubation promptly, we employ the use of two digital timers (Polder 898-90 Clock, Timer and Stopwatch). Both timers are set to the same time, one remains in the lab on top

of the incubator (Fig. 1C), and one goes with the responsible party (the timer came with a lanyard, Fig. 1D). Ultimately, the person wearing the second timer takes responsibility for removing the tissue at the appropriate time. That said, the timer in the lab acts as a safety net. When it goes off, if someone is already in the evaluation room, they can alert the responsible party. This redundancy has worked to our benefit at least once when the battery died in the wearable timer.



Figure 1D An eye bank technician keeps the 2nd timer close at all times to minimize the possibility of over-warming corneas.

warming protocol. In addition, eye banks currently do not have a standardized method of tracking and communicating such warming times to each other for tissues that are exported/imported. Eye banks should consider the possibility of reporting incubation times, cycles, and temperature on the tissue report so that importing eye banks can determine if additional warming can be done safely. This is particularly relevant to importing and processing eye banks. Since suitability has already been determined, a full incubation cycle would not be needed, although it would be helpful to warm the tissue enough to speed up the pre-processing evaluation time. Without knowing how many times, for how long, and at what temperature a tissue may have been incubated, it is impossible to know the impact of an additional warming cycle. We routinely perform an evaluation of imported corneas prior to processing to ensure that no damage occurred during shipping. Therefore, the ability to safely re-warm imported corneas could speed up this process and allow eye banks to spend more time focused on processing and post-processing evaluation while still meeting courier pick-up deadlines.

It is also our intention to work with other eye banks to standardize a method for tissue incubation. Currently, only limited incubation conditions have been tested and proven to be safe, so it is important not to over incubate corneas and inadvertently increase pathogen growth or risk damaging the endothelium, especially for imported tissue. We do not currently incubate imported tissue for this very reason. By standardizing warming protocols, or at least having the ability to communicate incubation parameters between eye banks (time and temperature), we can work together to increase tissue safety and improve efficiency as an industry.

A major benefit of rapid corneal warming for eye banks is the improvement in process efficiency for tissue evaluation. It has allowed evaluations to be scheduled and shorten the total time tissue is out of the refrigerator.⁶ We can now be confident that corneas are ready to be fully evaluated in a predictable fashion. An example of when this predictability is helpful is in cases where the endothelium appears to have an inverted appearance even after incubation for 1.5 hours, which still occurs on occasion. In our experience, the endothelium does not look better even after a second cycle of incubation. This suggests that we can confidently proceed with evaluation after a 1.5 hour warming period. We no longer wait, hope, and wonder if the corneas will look better if we give it more time to warm, as we now know that the endothelium tends to look as good as it can look after the first warming cycle.

RESULTS

Qualification of the new incubator was completed in 3.5 weeks. Initial temperature stability tests showed that the incubator maintained an average temperature of $35.1 \pm 0.3^{\circ}\text{C}$ (range: $34.8 - 35.8^{\circ}\text{C}$) during an 8-hour testing period. At this temperature, cold Optisol-GS (20mL) stored inside of Krolman viewing chambers (Krolman, Boston, US) were warmed to an average temperature of $34.3 \pm 0.4^{\circ}\text{C}$ (N=5, range: $33.7 - 34.7^{\circ}\text{C}$) after 2 hours of incubation. Both tests successfully satisfied the predetermined 'passing' parameters.

DISCUSSION

We have implemented and utilized our warming protocol over the past year without incident, and have warmed, evaluated, and distributed over 1700 corneas for transplantation. We have not observed an increase in adverse reactions reported in surgeon follow up questionnaires, nor has there been an increase in occurrences of primary graft failures. Thus, warming donor corneas for the purpose of tissue evaluation in a very regimented manner can be done safely. Below, we discuss some key issues – that we learned during our process, and as we look to the future – that deserves careful consideration.

The Eye Bank Association of America does not currently have standards governing tissue warming times, and there are no resources available to help eye banks implement this

Some other considerations (a.k.a. questions we've been asked)

Work flow: How do we determine how many corneas to incubate at one time?

Tissue evaluation takes a high priority, with the general consensus being that newly recovered corneas should be evaluated as soon as possible. With the recommendation that the incubation cycle should not be interrupted by adding or removing corneas mid-cycle, it is ideal to maximize the number of corneas that goes into each cycle (keeping in mind positioning guidelines discussed earlier). For us, this sometimes means waiting until expected incoming tissue arrives before incubating tissue already in house. Alternatively, if tissue demand is high and timelines tight, waiting for incoming tissue may not be the most effective choice. Adjustments to incubation cycle volume should be made to accommodate factors such as tissue needs and technician schedules. Generally, staffing tends to differ in the morning and evening, and weekdays and weekends. So, at times it may be unreasonable to load numerous corneas into one cycle if staffing is not appropriate to complete corneal evaluations in a reasonable time frame before returning them to cold storage. Because incubator implementation has led to the ability to predict when corneas will be ready for specular microscopy, the total time required for full tissue evaluation (specular, OCT, and slit lamp) can be better predicted. Thus, technicians are now better able to gauge how much time in their schedule to allot for evaluations, resulting in a more fluid and efficient evaluation process that can be modified based on the number of corneas that can be evaluated per cycle.

Who can incubate corneas? Is it limited?

Due to corneal incubation being a new process, it was important that we established control over the process. Prior to incubator implementation, multiple departments were capturing and evaluating specular images, including but not limited to: recovery technicians, processing technicians, and distribution staff. One of the advantages of this was being able to get specular evaluations done after hours. This did have its set-backs, as some of the staff that were performing specular evaluations were not trained on OCT and slit-lamp. This usually means these corneas undergo an additional warming cycle the following morning to complete tissue evaluation.

As we considered the implementation of rapid warming using an incubator, we reevaluated this process. With an emphasis on process control, reducing time out of the

refrigerator and number of warming cycles, and completing the full evaluation, the decision was made to only train staff that perform complete tissue evaluations. This has reduced the number of staff performing specular microscopy but has not negatively impacted work flow or efficiency. The predictability of tissue readiness has actually increased work flow efficiency. Because trained staff can complete tissue evaluations (OCT and slit lamp exam) in one sitting, the majority of incubated corneas get a full evaluation after one warming cycle. Additionally, with fewer staff performing specular evaluations, there is likely more consistency in these measurements.

What is the warming schedule like?

As mentioned previously, one of the major advantages of incubation is the shortened and predictable timeframe in which corneas reach optimal temperature for specular imaging. The minimized guesswork transcends into the scheduling realm, as technician work flow is no longer interrupted by checking on the readiness of corneas that have been sitting out. While tissue evaluation is a high priority, it is likely that staff's time is split between tissue evaluation and other tasks. With rapid warming via an incubator, the evaluation process can be better controlled and tailored to technician schedules, rather than other tasks having to be shifted due to the unpredictable corneal ready times.

Knowing just when a cornea will be ready for specular imaging allows for improved efficiency and planning. Eye banks can set up an incubation schedule that best fits their needs and staffing. Technicians themselves can also modify evaluations based on individuals' daily schedules. For example, if multiple pairs of corneas are checked-in near the end of a shift, the technician can incubate the appropriate number of corneas knowing approximately how long evaluation will take. This reduces the chances of the technician staying overtime, or corneas being subjected to multiple warming cycles.

CONCLUSION

We are sharing our experience with implementing an incubator and a tissue warming protocol to provide other eye banks a standardized method that has been proven to be safe and effective. Tissue incubation for the purpose of evaluation has significantly improved our image quality and evaluation process. In order to safely implement these improvements as an industry, it is important that eye banks are transparent about their warming protocols. Details of

warming cycles should be shared with importing or processing eye banks, so the safety of additional warming can be evaluated.

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