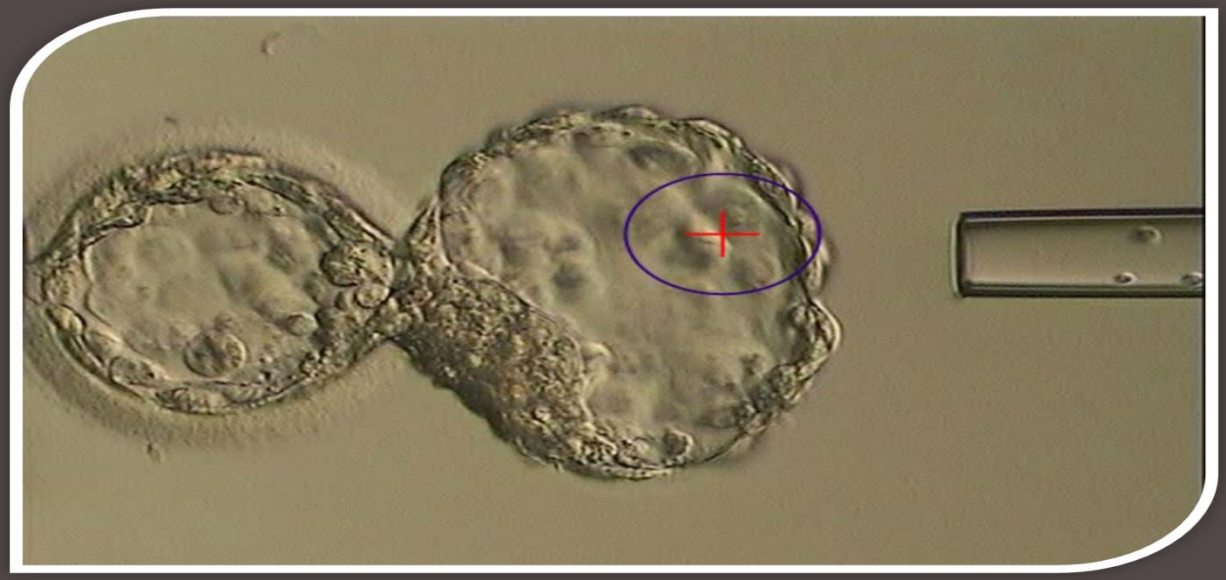
TROUBLESHOOTING PGT



2018 CRB Workshop

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PREIMPLANTATION GENETIC TESTING (PGT)

New names for PGD and PGS

The terms PGD and PGS are being replaced by new terminology in the International Glossary on Infertility and Fertility Care.

A few months ago, the **International Glossary on Infertility and Fertility Care** published a revision to provide the medical and scientific communities with a consensus set of terms and definitions that can be used globally to ensure consistency in registering specific fertility care interventions.

In this revision, the terms PGD and PGS are replaced now by new definitions.[1]

The new name for all tests is **Preimplantation Genetic Testing (PGT)**.

This includes:

- **PGT for aneuploidies (PGT-A)** – Previously PGS
- **PGT for monogenic/single gene defects (PGT-M)** – Previously PGD
- **PGT for chromosomal structural rearrangements (PGT-SR)** – Previously PGS translocation

WHAT IS TROUBLESHOOTING?

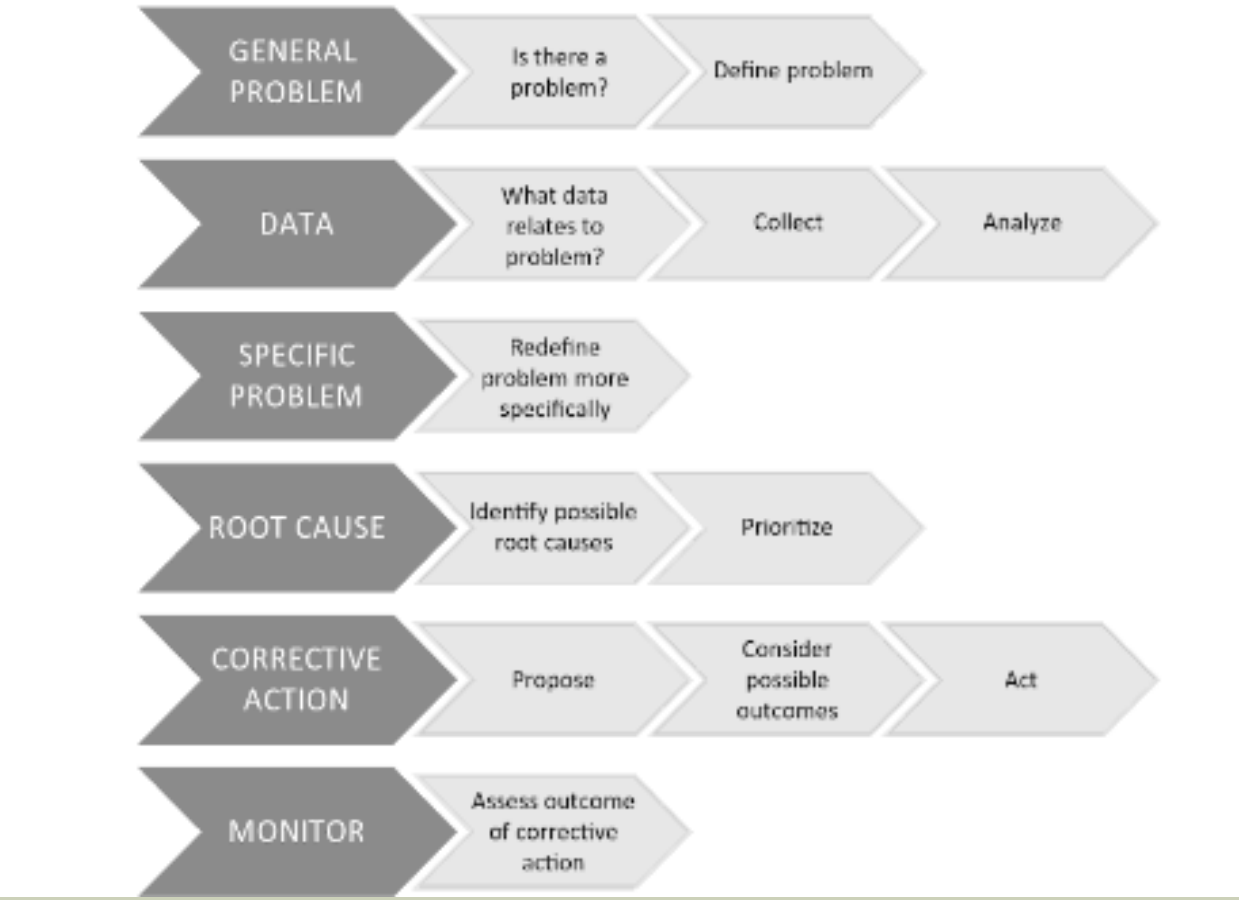
Planning ahead is always a great goal but we all should learn how to TROUBLESHOOT a problem in the laboratory

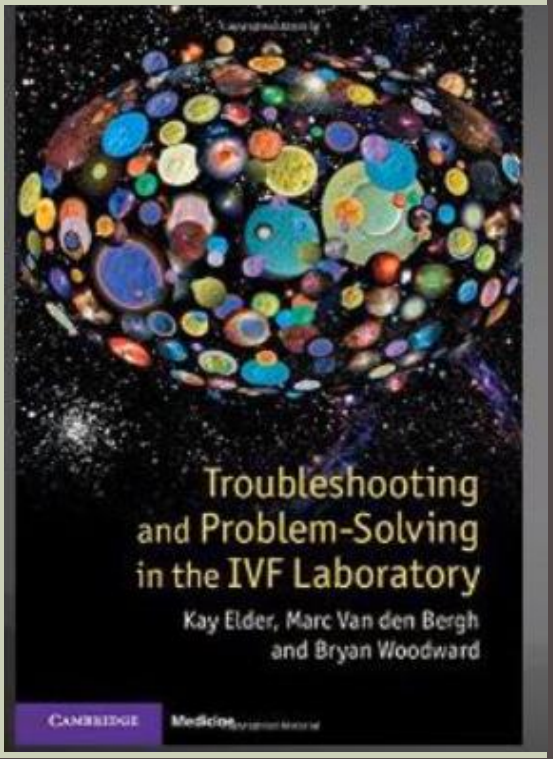
The five-step troubleshooting process consists of the following:

- Verify that a problem actually exists.
- Isolate the cause of the problem.
- Correct the cause of the problem.
- Verify that the problem has been corrected.
- Follow up to prevent future problems.

TROUBLESHOOTING

Six Step Process described by Kay Elder, et al.





TROUBLESHOOTING ISSUES ASSOCIATED WITH PGT IN THE IVF LAB

- Biopsy of Embryos
- Tubing Samples
- Shipping Kits
- Sample results / PGT Reports

BIOPSY OF EMBRYOS

Day 5 biopsy has been adopted by many IVF labs but what are some of the main issues that arise from this technique?

- **Equipment/Supply Failures:**
  - laser does not work
  - No biopsy needles in the lab
  - No kits/buffers in the lab
- **Procedural Failures:**
  - Sticky biopsy pieces
  - ICM hatching out of zona on Day 5
  - Accidental chimeras (2 blasts grow together)

EQUIPMENT/SUPPLY FAILURES:
LASER IS NOT FIRING!

INSTANT FIX

- Reboot to reload laser software.
- Check all laser cables, tighten all connections at the back of computer and laser box.
- Check with IT to make sure there wasn't any updates that interfered with the laser software
- Call the manufacturer

Last Actions

- Biopsy embryos the next day
- Freeze blastocyst – Thaw/Biopsy later

Preventative/ Corrective Action Based on Troubleshooting

- Daily laser QC + quarterly laser software maintenance + annual laser software/hardware maintenance
- Purchase a 2nd laser setup
- IT department does not update the "Laser" computer without permission
- Learn the "Rub or Flicking" technique for biopsy without laser firing

EQUIPMENT/SUPPLY FAILURES: NO BIOPSY NEEDLES, KITS OR BUFFERS!

INSTANT FIX

- Call a local IVF lab – beg for supplies
- Call genetics lab – how soon can kits/buffers be sent – overnight?
- Call genetics lab – can you use a completely different kit/buffer?

Last Actions

- Biopsy embryos the next day
- Freeze blastocysts –Thaw/Biopsy later

Preventative/ Corrective Action Based on Troubleshooting

- Setup auto-supply (standing) orders with genetic labs for kits/buffers
- Setup auto-supply (standing) orders for PGS supplies (biopsy needles and holding needles).
- Ask the genetics lab if they have ever run samples in buffers from other competitors = contingency plan.
- Weekly monitor of all PGT related supplies.

KIT EXCHANGES THAT "MAY" WORK

- Most buffers are a basic solution so plain media might work (PBS).
- Certain large genetic companies have accepted kits from others:
  - In the case of no maternal or paternal DNA available, samples initially placed in Natera kits with buffers were sent to Igenomix for testing (no requirement for parental DNA)
  - In the case of cousin marriages (info disclosed AFTER the tubing)

FULL DISCLOSURE = NO RESULTS ARE PROMISED, THIS IS A RISK!!

PROCEDURAL FAILURES: STICKY BIOPSY SAMPLES

INSTANT FIX

Biopsy sample is stuck to the outside of biopsy needle

- Tap the needle holder sharply to cause the needle to bounce in the media = freed tissue piece
- Gently but quickly aspirate the biopsied sample back and forth into the biopsy needle until the piece slides easily out of the needle
- Use holding needle to gently aspirate biopsied sample, release it from the biopsy needle

Last Actions

- Re-set the biopsy needle, biopsy the embryo again

Preventative/ Corrective Action Based on Troubleshooting

- Weekly check of PVP supply
- Make all biopsy dishes with 5 to 10 uL PVP bubble
- Follow the Biopsy SOP: Fire only between cells, the less cellular damage = no sticky pieces
- Follow the Biopsy SOP: Fire only 3 to 4 times, use manual pulling force to tear biopsied tissue from blastocyst

PROCEDURAL FAILURES: ICM HATCHES FIRST

INSTANT FIX

- Reposition the blastocyst away from the hatching ICM, make a new opening in the zona and biopsy the embryo

Last Actions

- Pull the entire embryo out of the zona, hold onto the trophectoderm (not the ICM) and then biopsy the hatched blastocyst.

Preventative/ Corrective Action Based on Troubleshooting

- Follow the revised Biopsy SOP: No AH of Day 3 embryos, hatch on Day 4. Wait until Day 4, AH the trophectoderm on the opposite side of the ICM
- Follow the revised Biopsy SOP: No AH of Day 3 embryos, breach and biopsy the embryo at the same time on Day 5, 6, or 7.

TUBING SAMPLES

During sample tubing the majority of issues are related to procedure:

- **Procedural Failures:**
  - Loss of sample piece
  - Extra buffer in the tube
  - Wrong buffer in the tube
  - Wrong kit used
  - Sample in the wrong tube (#5 in tube #9)

SHIPPING KITS

Shipping involves procedure failures as well:

- **Procedural Failures:**
  - No courier ordered
  - Courier does not come to the lab for pickup
  - No dry ice for shipping kit

SAMPLE RESULTS/ PGT REPORTS

Sample results and PGT Reports are the most critical to this technique, it is how you will proceed with embryo transfer and the treatment plan for your patient, so you want to be sure they are correct based on sound technique.

Failures in this area will have severe impact, and are usually from procedural failures:

- during the creation of the embryos = wrong egg or sperm
- biopsy of the embryos = wrong embryo biopsied
- tubing of the samples = wrong number on tube, does not match embryo
- during cryopreservation = embryo placed on an incorrectly labeled device

DON'T DESPAIR

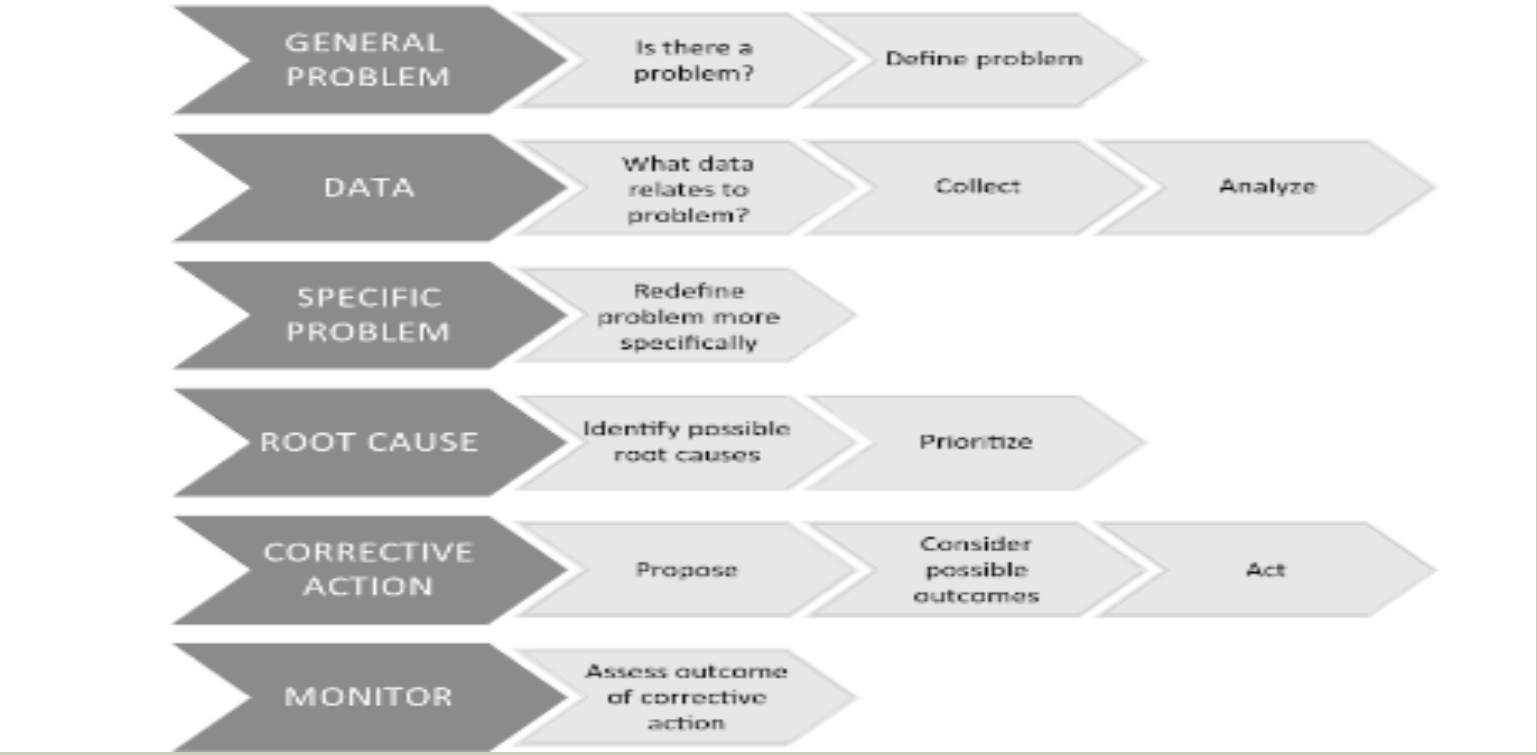
Before Troubleshooting Workshop

After Troubleshooting Workshop

TROUBLESHOOTING

Learn how to build your troubleshooting flowsheets to solve your PGT lab problems and errors.

There are 6 main steps to help troubleshoot any laboratory issue.

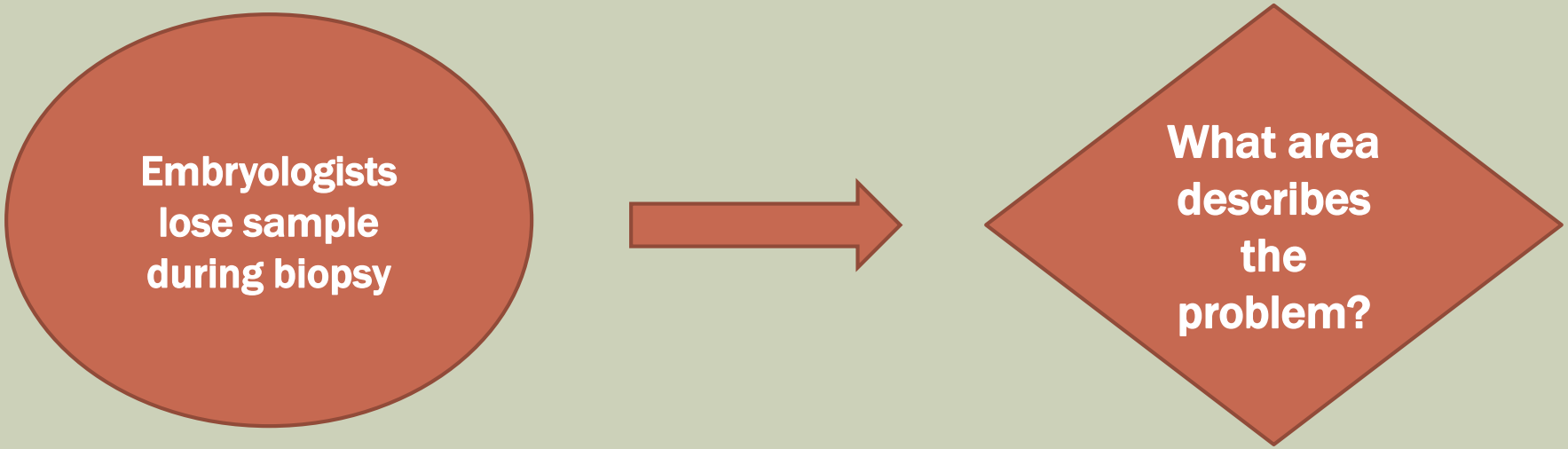


TROUBLESHOOTING – STEP 1

First, you should start by defining your MAIN PROBLEM.

TROUBLESHOOTING – STEP 1

Defining the problem



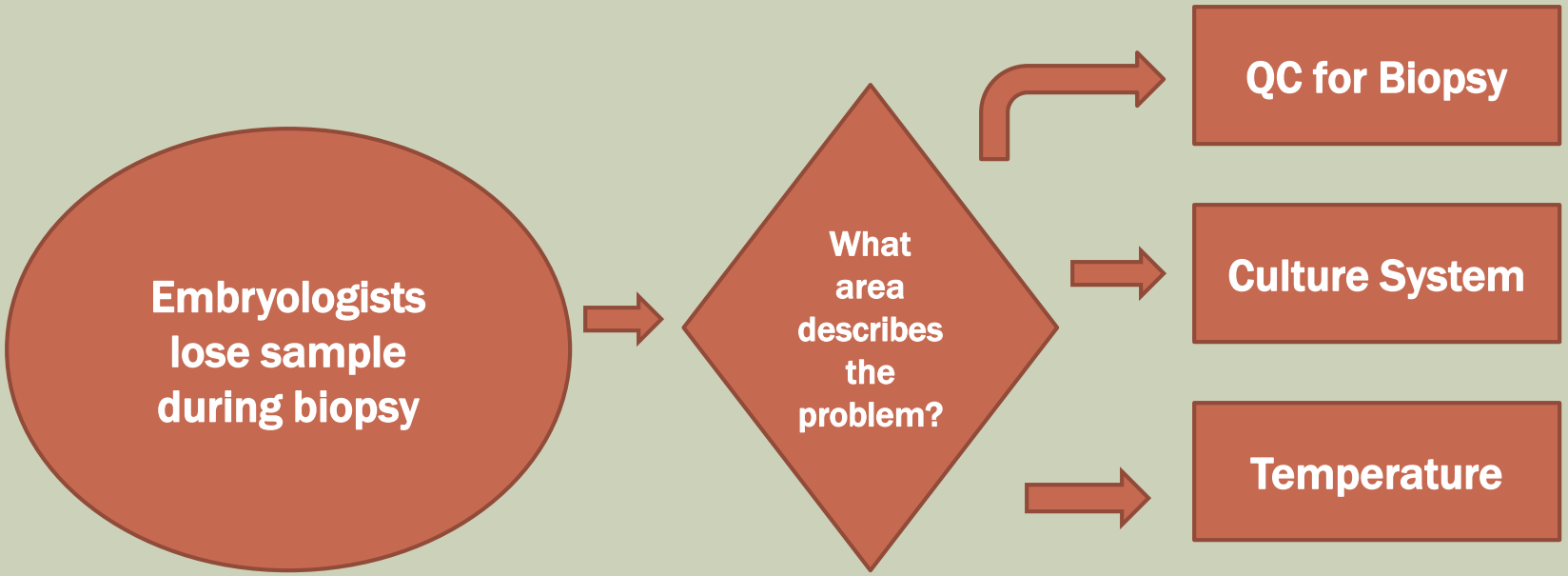
TROUBLESHOOTING – STEP 2

Gathering all data or information that relates to the problem

Possible CLUES?

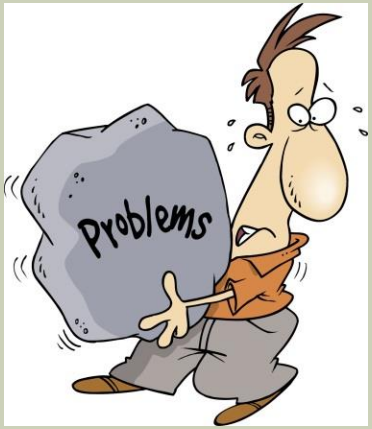
TROUBLESHOOTING – STEP 2

Defining the problem



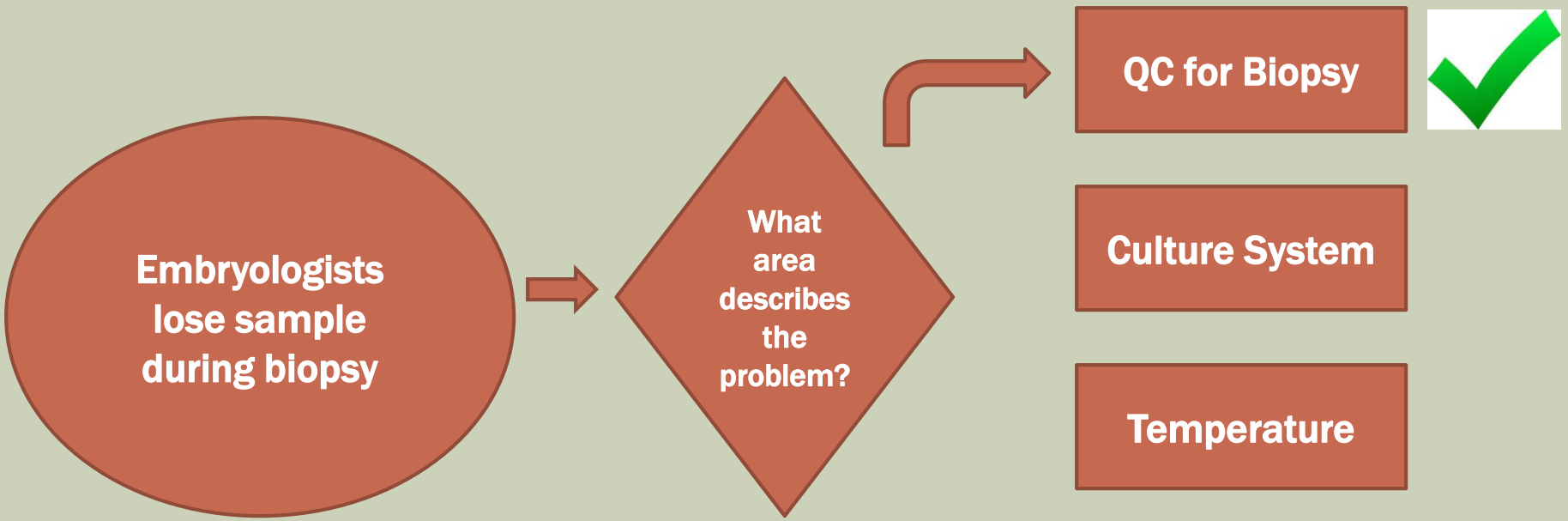
TROUBLESHOOTING – STEP 3

Look at only information specific to this problem



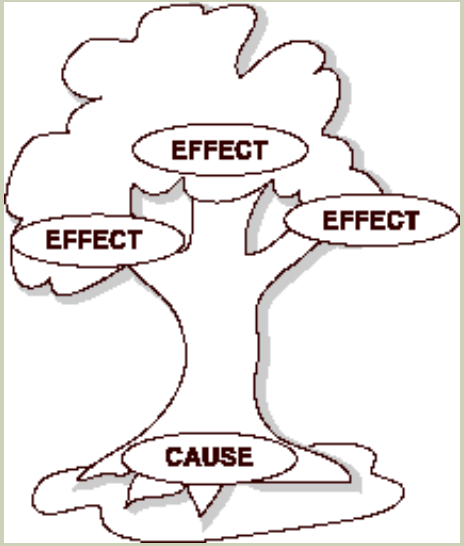
TROUBLESHOOTING – STEP 3

Defining the problem

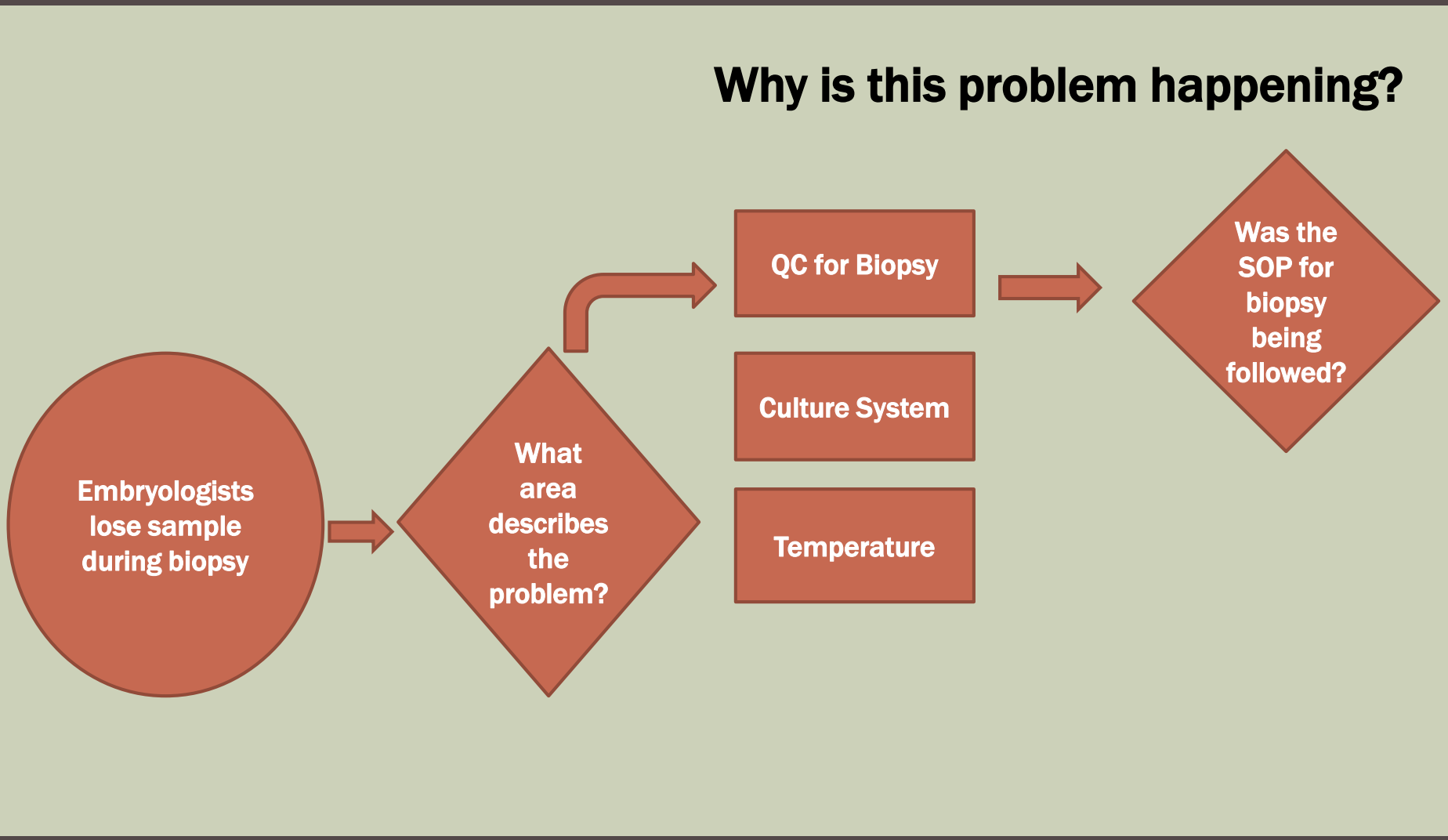


TROUBLESHOOTING – STEP 4

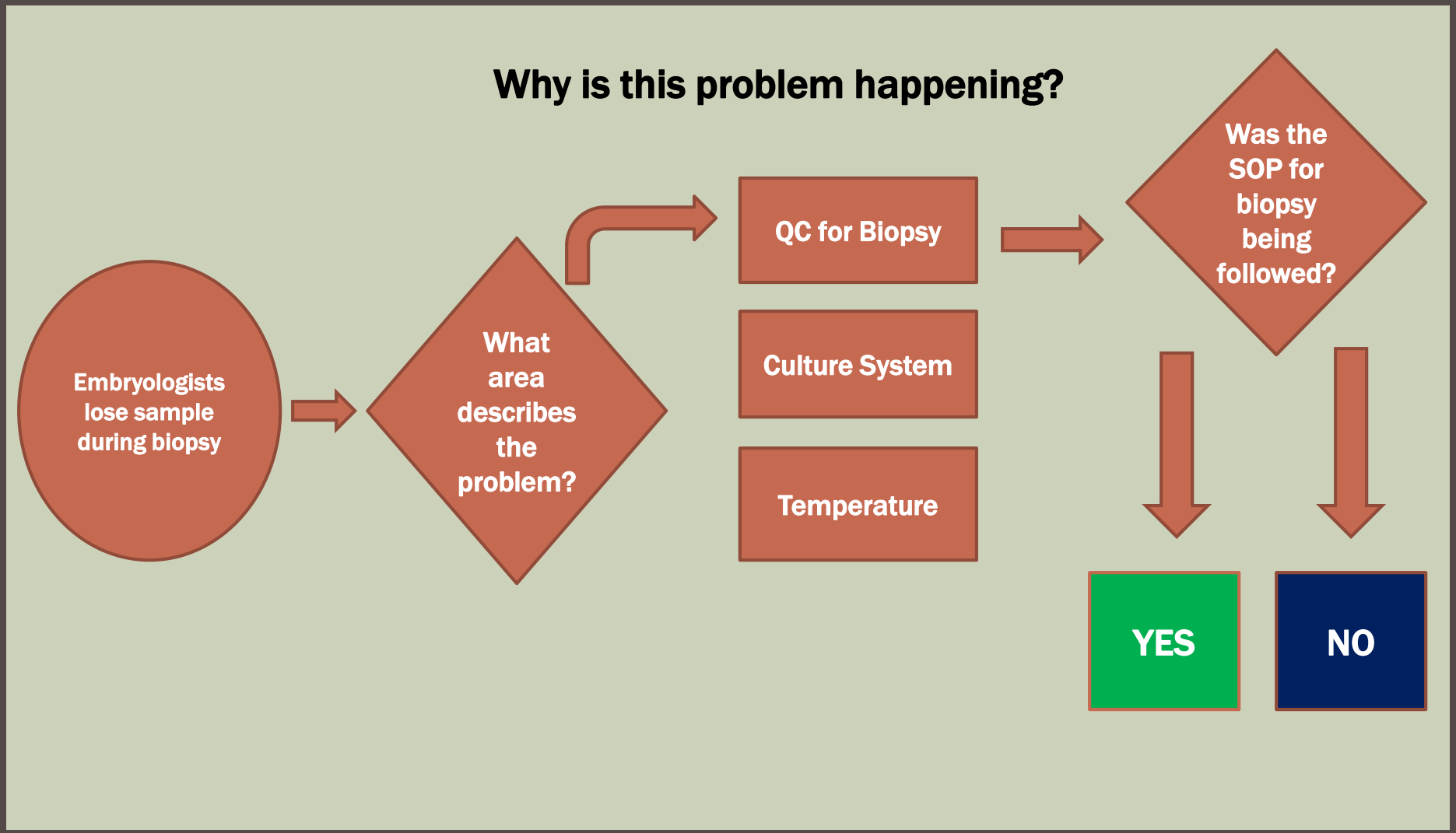
Why is this problem happening?



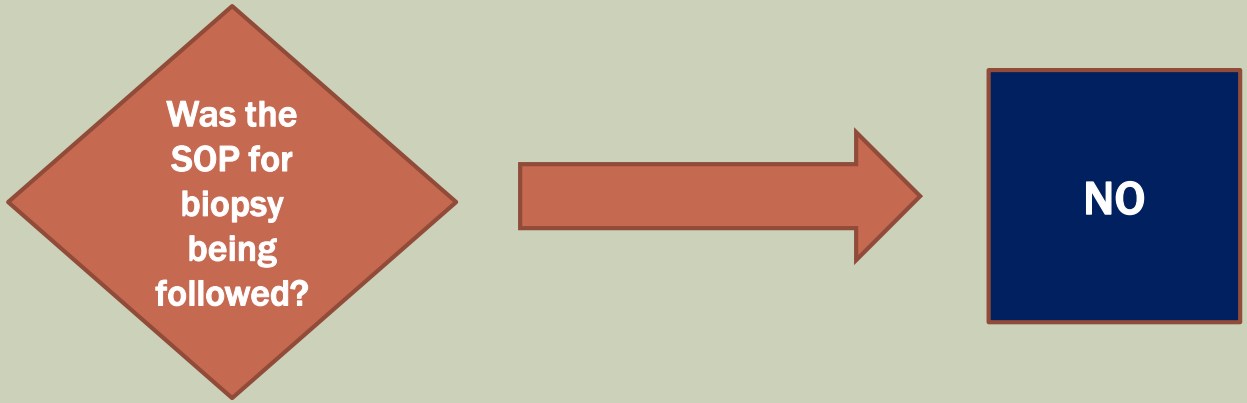
TROUBLESHOOTING- STEP 4



TROUBLESHOOTING – STEP 4

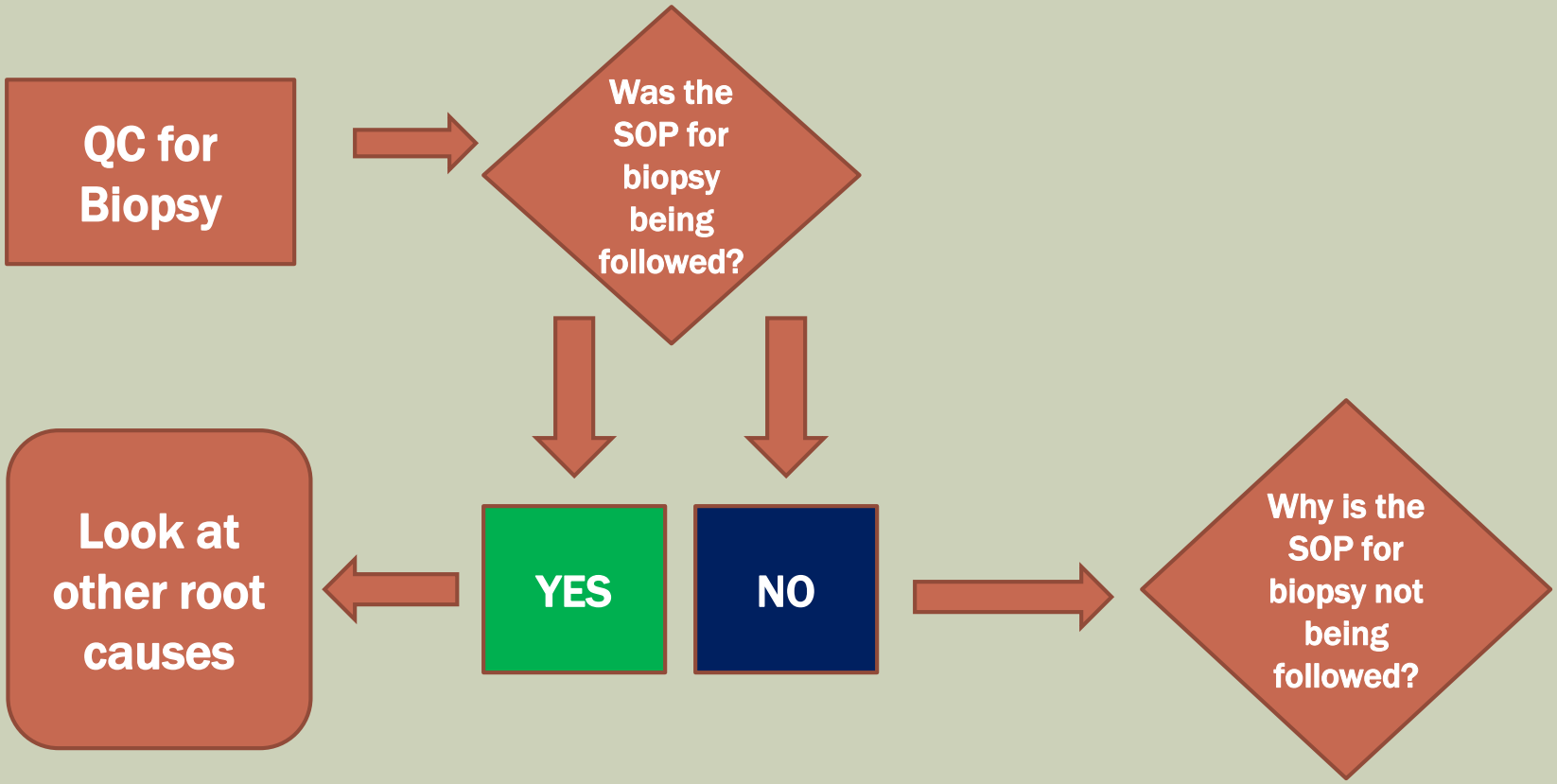


TROUBLESHOOTING – STEP 5

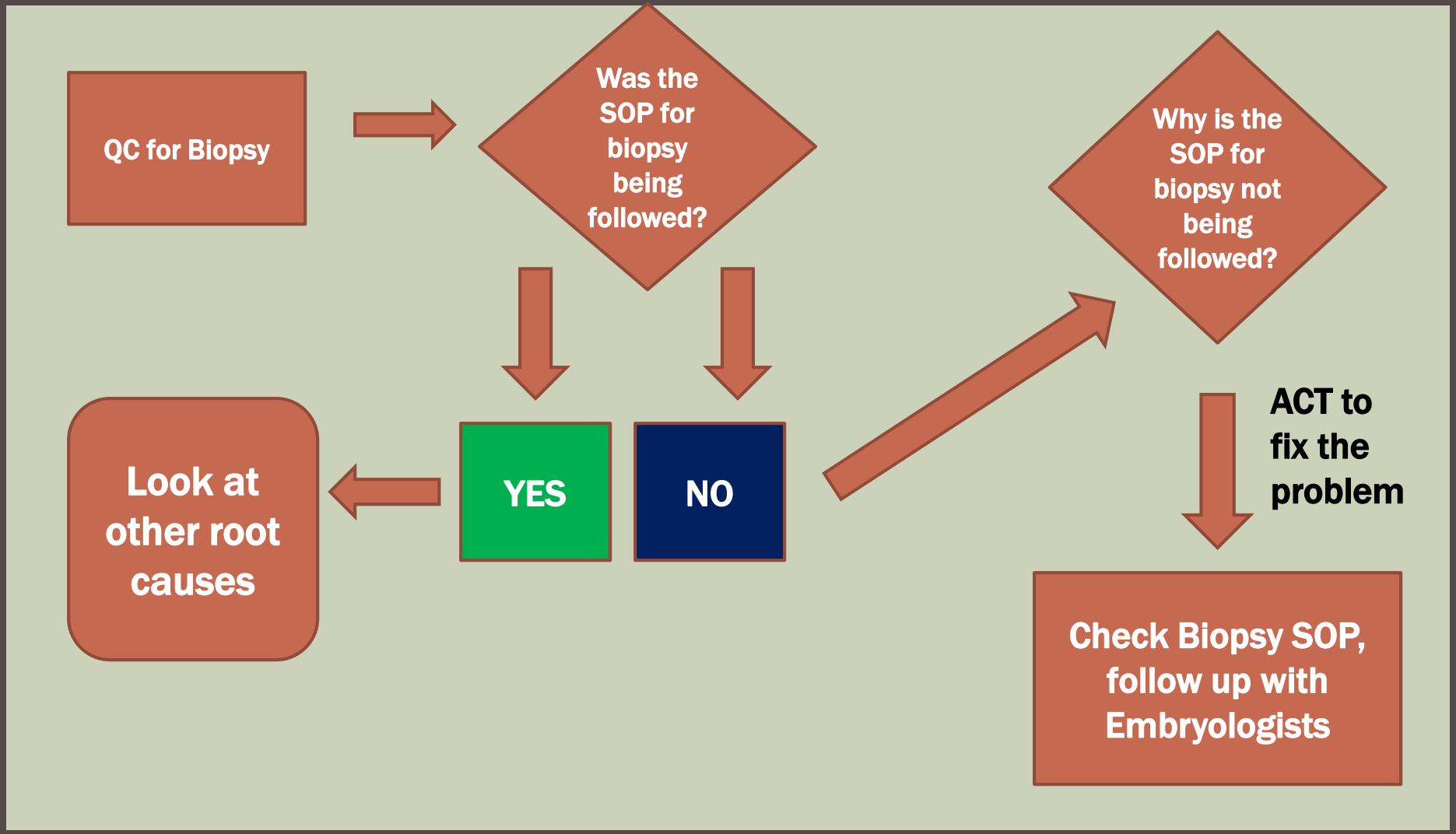


How should you fix this specific problem?

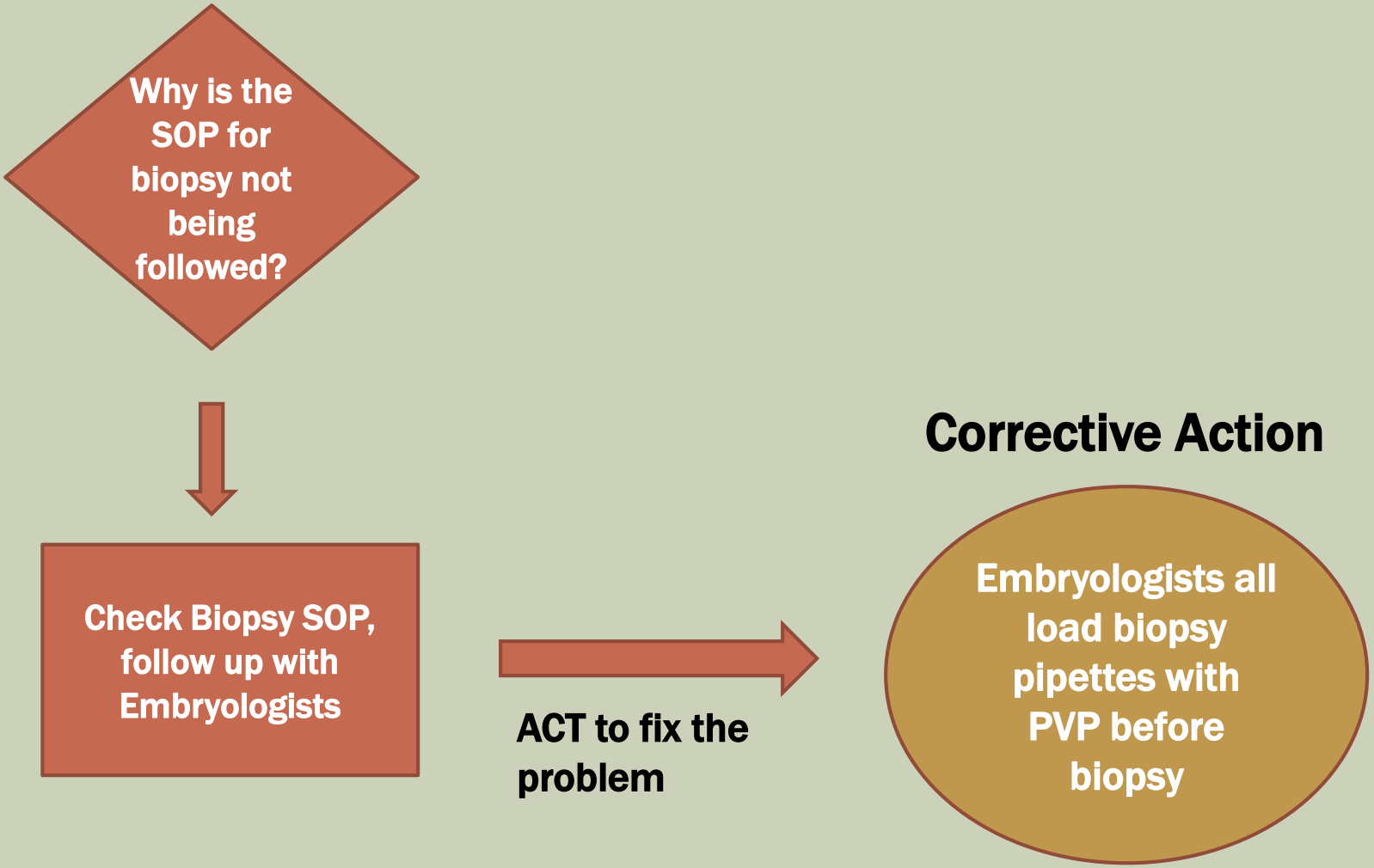
TROUBLESHOOTING – STEP 5



TROUBLESHOOTING – STEP 5



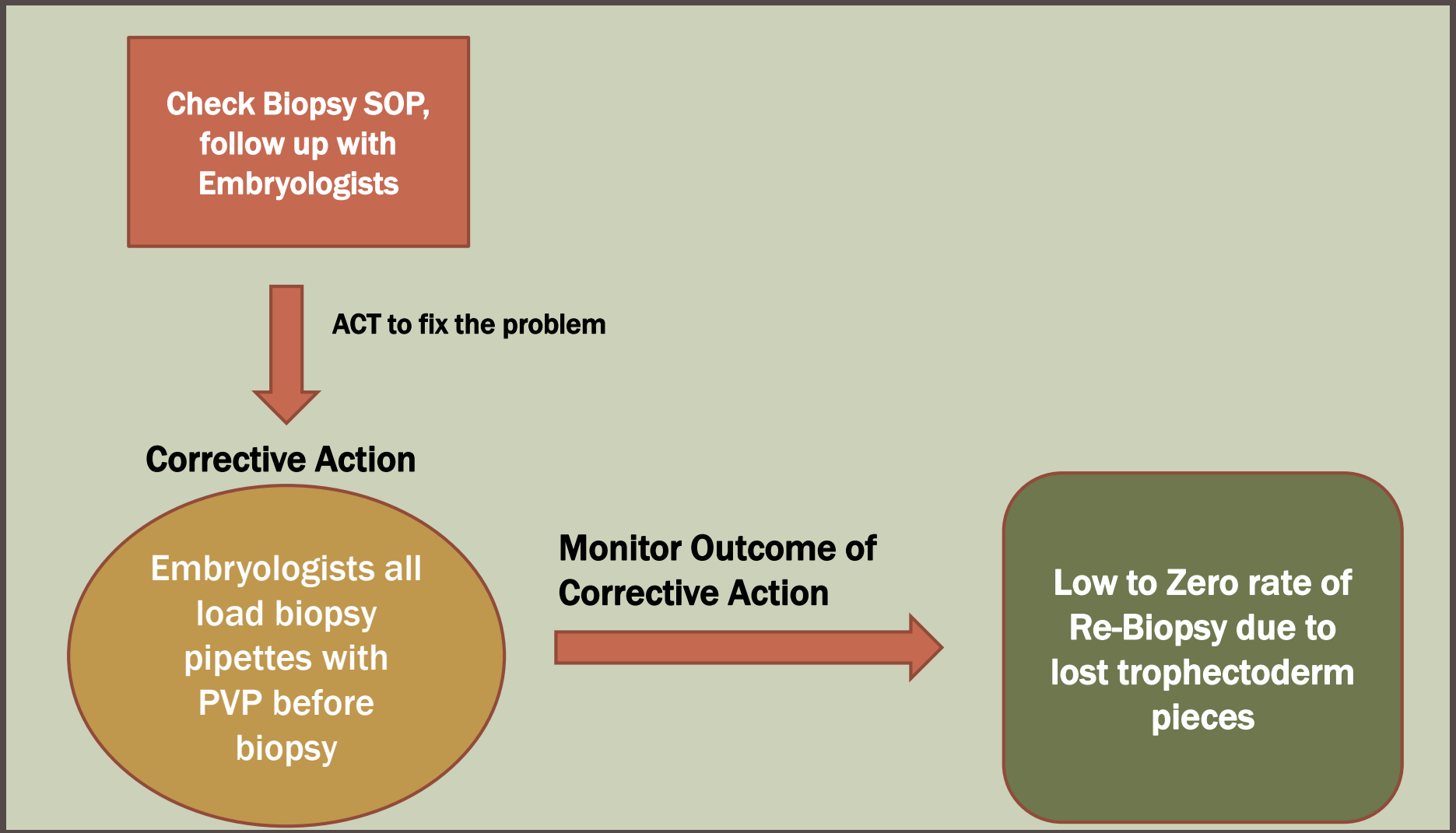
TROUBLESHOOTING – STEP 5



TROUBLESHOOTING – STEP 6

Did the Corrective Action fix the problem, any reoccurrences?

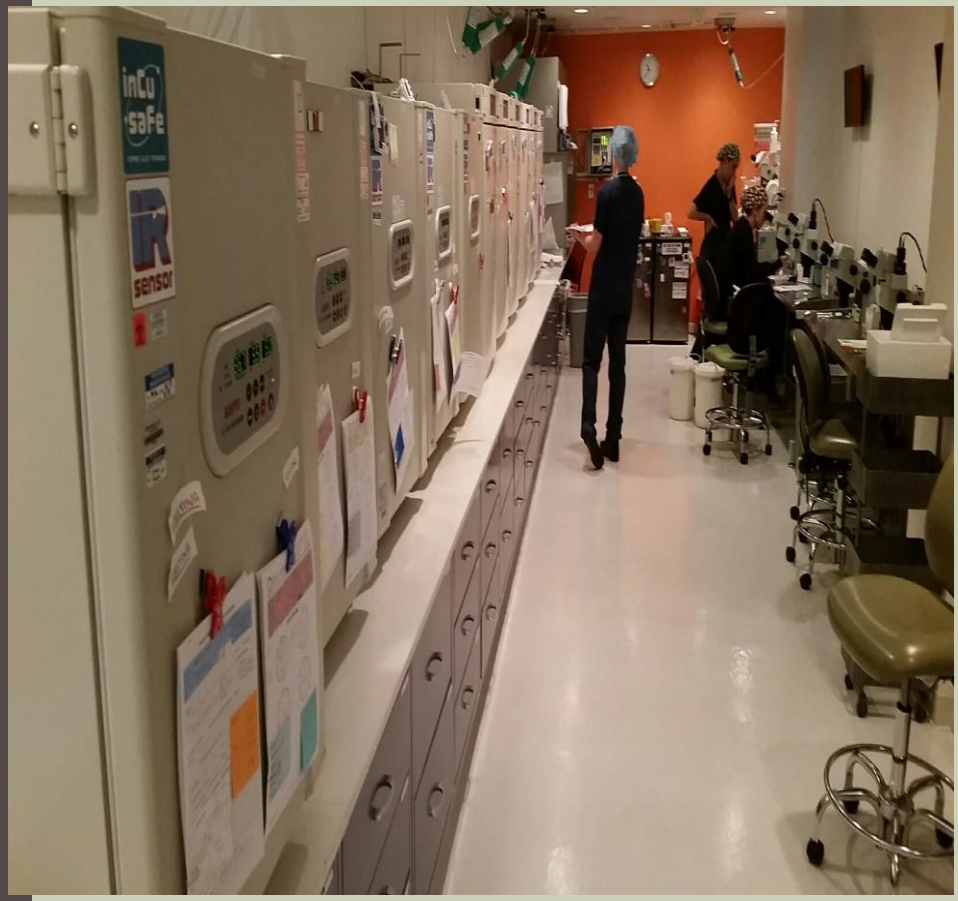
TROUBLESHOOTING – STEP 6



TROUBLESHOOTING "NO RESULTS" RATE

- **Identify Main Problem**: The No RESULTS RATE is HIGH from the genetics lab
- **Identify data/areas that relate to the Main Problem**:
  1) Embryologists = Biopsy procedure
     Tubing procedure
     Shipping procedure
  2) Genetics Lab = Expected "No Result" reporting rates
- **Prioritize + Analyze data**: What is the "No result" rate by Embryologist?
- **Redefine Problem Specifically**: The "No Result" rate is different among Tubing embryologists
- **Find the Root Cause**: Check the Tubing SOP, Follow up with Tubers
- **Take Corrective Action**: Re-Train Tubers with the highest "No Result" rate
- **Monitor the Problem**: Lowered "No results" rates reported?

IVF LAB LAYOUT – A QUIET SPACE FOR BIOPSY



HECTIC SPACE

QUIET SPACE

IVF LAB LAYOUT – A QUIET SPACE FOR BIOPSY

PGT LAB CORNER – Dissecting Scopes for Tubing

Primary PGT Scope with Laser – Isolette

EMBRYOLOGISTS – EQUIPMENT

Does your lab possess the necessary equipment for performing Trophectoderm Biopsy?

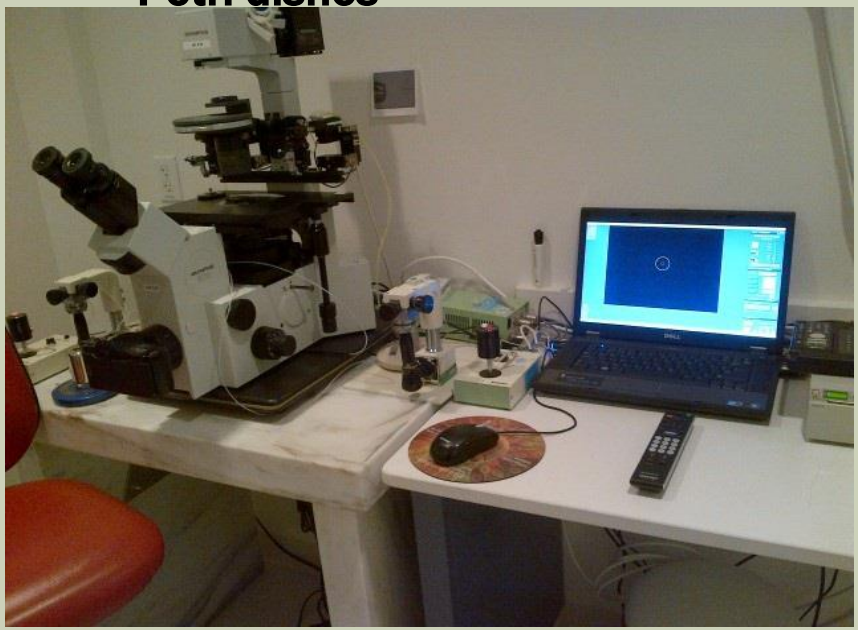
Inverted Microscope with manipulators
Laser – Saturn, Lykos, Zilos, Octax with foot pedal
Dissecting Microscope – tubing samples

Hood/Clean table space – tubing samples (no heated surfaces)
Benchtop, Isolette, or small incubator – holding embryos culture / biopsy

EMBRYOLOGISTS – INVENTORY/SUPPLIES

What new or increased supplies do you need to incorporate Trophectoderm biopsy?

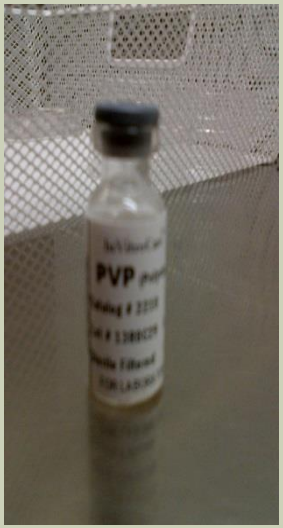
- **Welled Culture plates - GPS or Corral dishes**
- **Strippers/Drummond tips = 130 and 300**
- **Petri dishes**



Secondary PGD Scope with Laser

EMBRYOLOGISTS – INVENTORY/SUPPLIES

What new or increased supplies do you need to incorporate Trophectoderm biopsy?



Culture media and Ambient handling media – GMOPS, MHTF

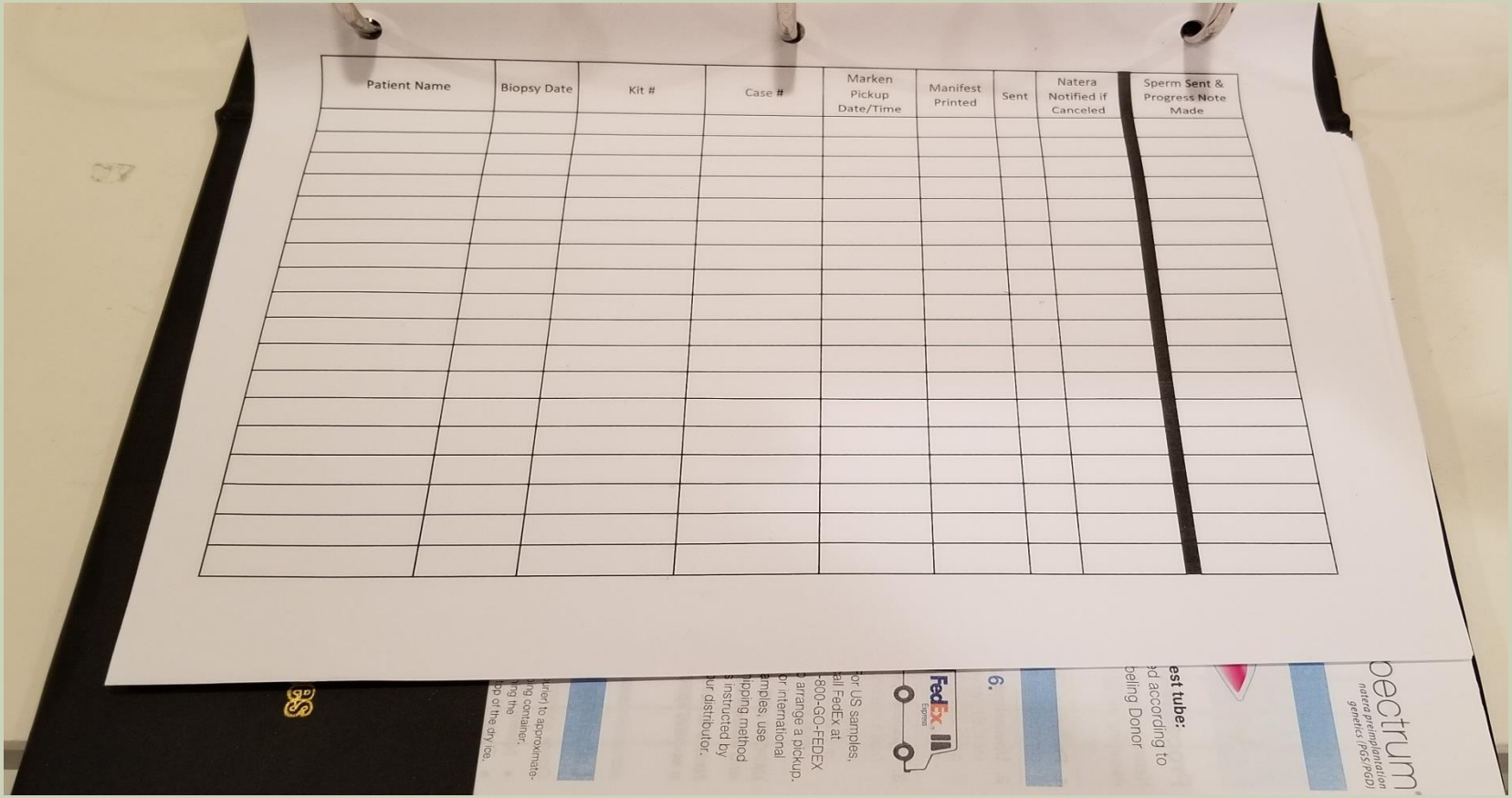
PVP - sample expulsion and to clean biopsy pipette

Cryo devices and Cryo media – more embryos to freeze!!

Storage – for biopsy kits and refrigeration of buffers

EMBRYOLOGISTS – SHIPPING LOGS

Keep a log of your upcoming biopsies and scheduled courier pickups



Troubleshooting PGT

THANK YOU!

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Pacific Fertility Center®