Quality Control– Lecture Eight
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Balancing Error Detection and False Rejection

First: Definition of Scientific Error (Definition of Error)

When a single measurement is compared to another single measurement of the same thing, the values are usually not identical. Differences between single measurements are due to error.

- Error: The difference between the measured value and the true value.

Second: Classification of Errors and Their Common Sources

Errors in scientific experiments and laboratories are divided into two main types, and each type has its own sources:

1. Random Error:

- Definition: An error that occurs due to chance and coincidence. There is always some degree of variability when making any measurement.
- Causes: Slight fluctuations in instrument performance, surrounding environmental changes, or the way measurements are read, which are errors that do not repeat with the same value every time.
- Scientific Solution: Scientists utilize the mechanism of replication (Replication). Replication is repeating the measurement many times and calculating its arithmetic average to reduce the impact of random error.

2. Systematic Error:

- Definition: An error that gives measurements that differ consistently and firmly from the true value in nature.
- Causes: It is often due to the presence of limitations or certain deficiencies in the used instruments or in the steps of the methodology itself.

The Common Sources of Error in general can be attributed to:

1. Instrumental Error: Occurs when the instruments being used are inaccurate, such as a laboratory balance that does not work correctly or is uncalibrated.

2. Environmental Error: Occurs when some factor in the surrounding environment (such as an unusual event) leads to a flaw in measurement. Like wind in the laboratory affecting the operation of the sensitive balance.
3. Procedural Error: Occurs when the researcher or technician does not commit to following the specific methodological protocol of the experiment.
4. Human Error: Is due to lack of attention (carelessness) or the natural limitations of human ability, and it is divided into two types:
    - a. Transcriptional Error: Occurs when data is recorded or written down incorrectly (such as writing a laboratory report incorrectly).
    - b. Estimation Error: Occurs when reading measurements on some instruments. Example: When reading a ruler, you may read the length of a pencil as 11.4 centimeters, while your colleague reads it as 11.3 centimeters.

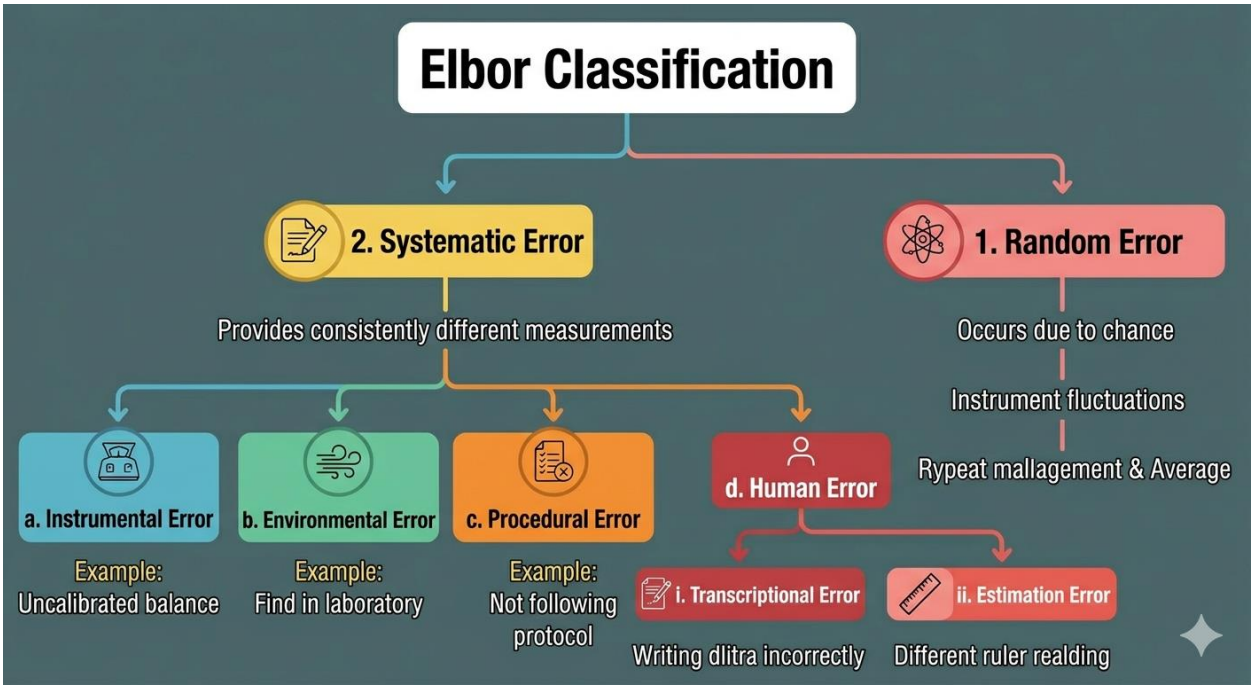

Diagram (1): Error Classification Tree

Third: Errors in Medical Laboratories (Errors in Medical Laboratory)

1. Types of Misdiagnosis Caused by Errors (Types of Errors)

- A. Miss diagnosis (Overdiagnosis): Diagnosing a healthy person with a disease they do not have. Example: Diagnosing a completely healthy person as diabetic.
- B. Delayed diagnosis: The diagnosis is correct and the results are accurate, but they are delayed in issuance and in reaching the doctor and patient, which affects the treatment plan.
- C. Missed diagnosis / Failure to diagnose: Total failure or inability to identify and diagnose the pathological condition.

2. The Sources of Errors in Medical Laboratories (The Sources of Errors)

- i. Pre-analytical Stage: Includes all steps and processes performed before the actual start of sample analysis. Examples: Labeling, sample collection process (Collection), choosing the correct tube (Correct tube), and drawing the correct volume for the sample (Correct volume).
- ii. Analytical Stage: Includes everything performed on the sample itself during the analysis process, whether that is inside the analysis instrument or during the fine transfer process by pipette (Pipetting).
- iii. Post-analytical Stage: Includes all processes following the completion of sample analysis. Examples: Writing final reports (Writing reports) and recording medical records (Records).

Sample Workflow & Error Sources Diagram

This diagram depicts the timeline of the sample's journey in the laboratory, from the pre-analytical stage to the post-analytical stage.

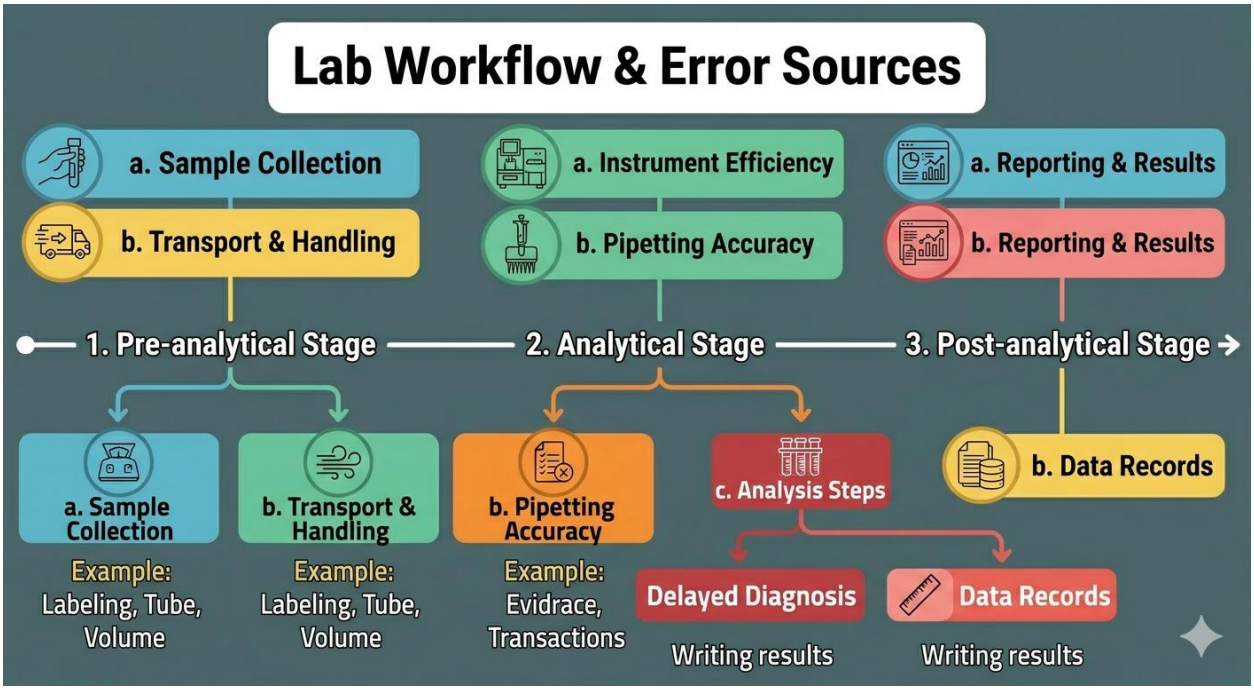

Diagram (2): Medical Sample Pathway and Laboratory Error Sources

Fourth: Troubleshooting Based on Quality Assurance Results (Troubleshoot based on Quality Assurance (QA) results)

- Troubleshooting: Is the process of determining the source of a systematic error and working to correct it. Professional Note: This process is considered an essential and necessary part of the laboratory profession. It is very important to take a step back and be deliberate when you are not sure of what to do for the next step.
- The Goal of the Troubleshooting Process: To determine why something does not work as expected and explain how to resolve the problem. Accurate descriptions of problems are the key and the essential beginning to finding the root cause.

The Six Typical Steps for Troubleshooting in the Laboratory:

1. Identify the problem: Spotting and defining the current problem clearly. Commitment to logical thinking.

2. List all possible explanations: Developing a list that includes all possibilities and explanations causing the problem.
3. Collect the data: Gathering data and reviewing controls (Controls) and protocols.
4. Eliminate some possible explanations: Comparing data and eliminating incorrect possibilities one by one.
5. Check with experimentation: Conducting a dedicated practical experiment to verify the remaining possibility (the final result).
6. Identify the cause accurately: And beginning processing and correction procedures.

This diagram represents a circular loop showing the six typical steps that must be followed for troubleshooting in the laboratory. This shape facilitates memorizing the steps and shows the top-down logical thinking for solving problems.

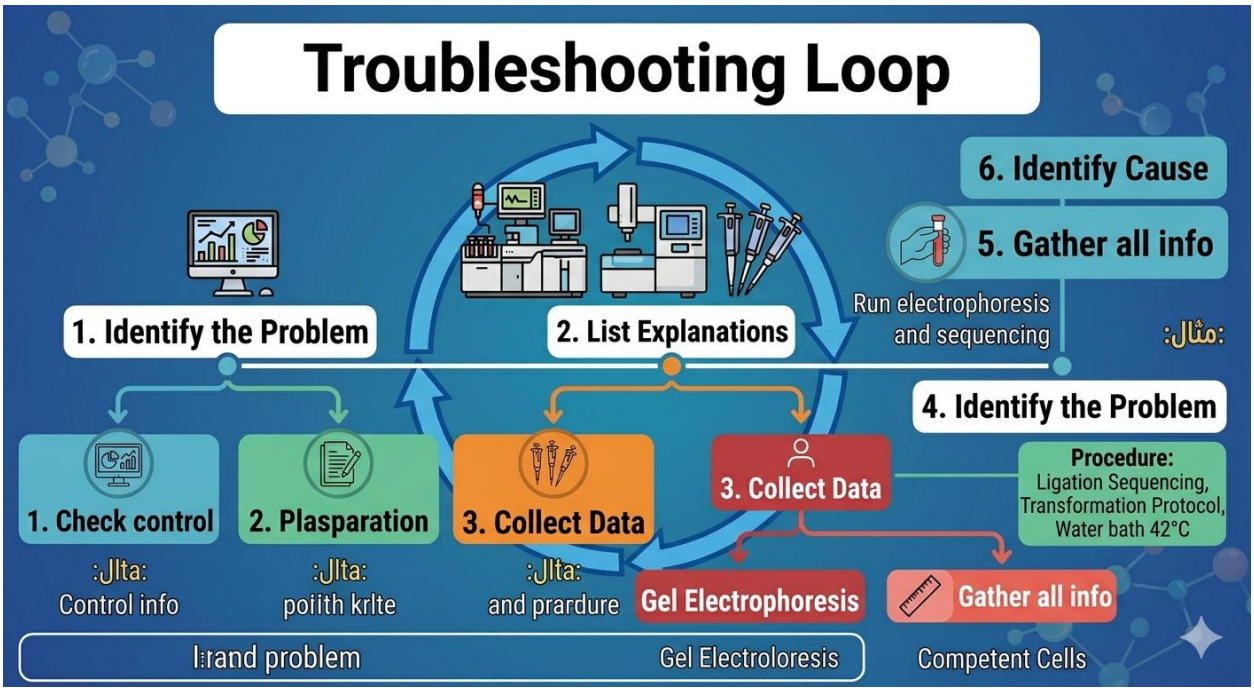

Diagram (3): Troubleshooting Loop

Fifth: Practical Case Study (Example)

Problem: No Clones Growing on the Agar Plate

Application of the six scientific steps to solve this biological laboratory problem:

1. Identify the Problem:

- Procedure: First, check all transformation plates (Transformation plates) and see if any colonies are growing on your control plates (Control plates).
- Conclusion: If there are colonies growing on your control plates, then the problem lies specifically in the transformation process of your plasmid DNA (Plasmid DNA).
    - Definition of Plasmid: It is a small circular DNA molecule found in bacteria and some other microscopic organisms.

2. List all possible explanations:

- After confirming that the problem does not lie in the competent cells (Competent cells) as a result of their growth on the control plate, the possible explanations for the cloning failure might be:
    - a. A flaw in the plasmid itself (Plasmid).
    - b. A flaw in the used antibiotic (Antibiotic).
    - c. A flaw in the temperature during the heat shock procedure (Heat shock procedure).

3. Collect the data:

- Reviewing Control Plates (Controls): If you included control plates in the transformation experiment, your positive control plate (Positive control) must contain cells transformed using an uncut control plasmid. This plate should contain many bacterial colonies. If only a few colonies grew, the efficiency of the competent cells (Competent cells) may be very low.
- Reviewing Procedures (Procedure): To know if the antibiotic is the cause, check whether you used the correct antibiotic for selection and at the

recommended concentration. To see if the incorrect temperature during the heat shock was the cause, check whether the water bath temperature was at 42°C.

4. Eliminate explanations:

- Based on the collected data, the following results appeared:
    - Many bacterial colonies grew on the positive control plate means the competent cells were efficient and sound (eliminating the cell efficiency hypothesis).
    - The correct antibiotic was used exactly at the recommended concentration : eliminating the antibiotic hypothesis as a cause of failure).
    - The water bath temperature was precisely set at 42°C the procedure was sound (eliminating the heat shock temperature flaw hypothesis).
- Logical Result: After eliminating all those factors, the only remaining possible cause is the plasmid DNA (Plasmid DNA).

5. Check with Experimentation:

- To test if the plasmid is indeed the problem, a gel electrophoresis check (Gel electrophoresis) is performed to ensure the plasmid is intact and not degraded, and that its concentration is not too low. In addition, the ligation process is verified via plasmid sequencing (Sequencing) to ensure that the insert DNA (Insert DNA) is actually inside the plasmid. And ensuring strict adherence to the transformation protocol regarding the required concentration.

6. Identify the cause:

- The last step is gathering information after running the verification experiments.
- Result: Sequencing data showed that there is no problem in the ligation process, but when looking at the gel electrophoresis (Gel electrophoresis)

results, a very faint band (Faint band) was noticed, which means that the plasmid concentration was extremely low.

- Final Diagnosis of the Problem: The main cause behind the failure of the bacterial transformation process is that the plasmid DNA concentration (Plasmid DNA concentration) was too low.