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Review Article

A Quality Improvement Cycle for Microbiology

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Abstract

Laboratory analysis is a process starting at the clinician (who orders a given exam) and ending at the same professional – when evaluating a result, then diagnosing and managing the patient [1]. That includes all microbiology lab determinations and other related complementary exams. From that standpoint, it works as a cycle, segmented into three main phases: pre-analytical, analytical and post-analytical [1-3]. Each period itself constitutes of different, successive tasks. All participants must be aware of that cycle if we expect the best results. Microbiology diagnosis requires the examination of all those steps in a general lab with the specificities that science. Extensive documentation of procedures, training of participants and verification of conformity if equipment's and inputs is needed at any good microbiology lab [4].

For the integral and most perfect management of the patient, each step must ground in a precise, high quality and conscientious execution. Errors at each task are possible, either human generated or produced by some automated process [5].

That requires evidence-based, quality-checked actions from each participant. For optimum performance and avoidance of re-working, the executors of each step must be attentive to the phases immediately before and after the current one, so that eventual failures are perceived early and dealt with [6,7].

All professionals must be aware of the possibility of failures — from the moment when the patient lives a clinician with an order for a given exam, until the moment he returns to the doctor for re-evaluation. In that context, therapeutic procedures and healthcare must work as a high reliability organisation, such as that of a precision industry [5,7]. While scrutinising each human or mechanical error before correction measures, we cannot neglect that the whole procedure must and avoid stating preventive measures to prevent future similar failure.

The consistent planning and monitoring of quality and its indicators result in continuous improving of quality and reliability [8]. That can be achieved, also in the microbiology Lab, through the so-called Demming cycle [9]: the continual sequence of Planning (improvement actions) [P], Doing (whatever is planned) [D] Checking (if everything is practised as planned) [C] and Acting (to correct deviations and promote further improvements [A] [8]. Grounding on that methodology, each good lab should establish some quality indicators (based on the most critical problems) and periodically submit to an audit process to determine what processes should be focused for improvements at the next phase [2].

Accordingly, physicians must be aware that the quality of the system begins and ends at their office. Therefore, if any doubts remain as to the clinical indication, sampling and processing, clinical significance of the results, it is mandatory to consult an adequate laboratory professional for enlightening.

Keywords: Quality; Microbiology; Laboratory

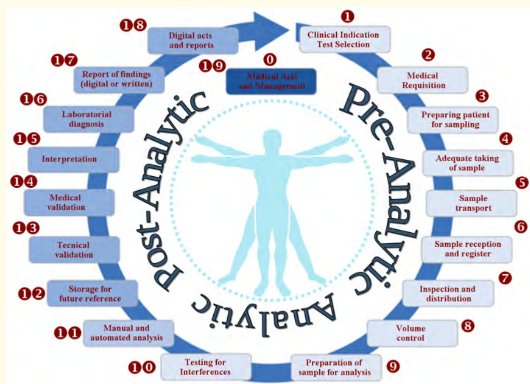

Figure 1: The cycle of integral management of the patient starts and ends at the clinician's office.

Introduction

A significant amount of the clinical decisions is ground in Laboratory results [10]. An integral commitment by all involved professionals is essential for the appropriate quality of a Clinical Laboratory and therefore for the correct diagnosis. When it comes to microbiology, that cannot be any different.

The cycle of analysis and patient management

All laboratory exams — including those of the Microbiology Lab — must go through a cycle that starts and ends at the clinician's office [1]. Laboratories can thus classify diagnostic tests into three main phases, classically named: Pre-analytic, Analytic and Post-Analytic [1,2]. As each stage can be the object of a particular set of possible errors, the process can be compared to the slicing of Swiss cheese—as each "hole" on a thin slice of the cheese may succeed another [7]. Whenever there is a hole in one or more successive slices of that hypothetical cheese, the resulting errors tend to become unavoidable — and the more severe they are as greater the number of holed slices. Modern laboratory theory, therefore, advocates that healthcare must be compared to high reliability organisations, such as a flying aeroplane, or an industry producing precision items such as components of computers or televisions [7]. As each layer (or stage of the diagnostic procedure) follows another, the individuals who perform each action shall be furthered to watch out — not only for the current stage but also for those immediately before or after it. The intention is to avoid small errors, or "fill each other holes of the cheese", so that while avoiding re-working, the complete procedure is exempt from the more significant errors that might occur without some automatic corrections at each level. Error control in healthcare must therefore rely on a systemic approach, recognising that human error is always possible but must be continuously prevented through control measures and methodological safeguards [7]. By continuously evaluating and analysing those gaps in quality of attention, it is possible to research and improve the processes [5]. For that, the complete documentation of the procedures using Standard Operating Procedures (SOP) is paramount [11]. Individual competences must also be attributed and accessed [12].

Figure 2: The Swiss cheese theory: whenever two or more slices of cheese show holes in the same place, there is a risk of propagation of an error situation.

The single stages of a clinical (and microbiological) diagnostic exams can be named as (Meyer, 2017):

Pre-analytical phase

- Clinical question and indications for the ordered tests;
- Selection of the test(s);
- Test request (order), either written or digitalised;
- Preparation of the patient for sampling;
- Taking the proper sample;
- Transport of samples to the central lab;
- Sample and medical order admission into the lab;
- Testing for suitability for analysis (sample inspection, sorting, volume control, interference testing etc.);

Preparation of the sample for analysis.

Analytical phase

- he analysis itself, ire., sub-sampling, selection of means for plating, incubation, input Into analysis automation etc.

Post-analytical phase

- Storage (e....g. of samples for an eventual counter-proof);
- Technical validation (ire., checking processes, analytical procedures and controls);
- Medical validation (ire., checking by any means if the results are clinically sound or possible);
- Interpretation (ire., evaluation of the results by a given professional);
- Laboratory findings (ire., checking other related lab results);
- Diagnosis delivery to the physician (by a given laboratory, written or digital): includes final evaluation of results—usually digitalised –, keeping records, preparing and reviewing reports; the final report must be assembled into an assembled report, communicating all findings, destined to the patient and the clinician.
- Digital (or written) file keeping. Includes maintaining all records related to a given exam in the lab (either printed or digital) and traceability of all procedures.

One must never forget the safe disposal of the biological, chemical and other residua — at each particular part of the process. It must be performed at most stages and immediately as they result from processing.

The task 3.4 (delivery of diagnosis) takes the patient (and results) back to the doctor, who will then clinically review the data, restarting the process as needed (with a new order, after diagnosing and managing the patient). Integral management of a given patient in all steps is dependent on comprehensive management and execution of all steps.

The pre-analytical tasks

A complete compendium of the pre-analytical phase would take a whole book. We can, however, make some comments as to the main determinants of quality at each of those steps [1].

The clinical question is paramount to the starting of the process. It should ground on the clinical context, predictability of a given disease, positive or negative predictive value of the exams, and diagnostics, prognostic or therapeutic utility. Any exam of unproven utility in that context should not be ordered at first [1].

Like the ancient Greek oracle: if one does not have a transparent inquiry, objectively expressed, the answer is correspondingly foggy. The experienced clinician must be able to determine what is the main diagnostic goal (i.e. to determine if some infection is present or probable; To stage a disease process; To be sure the patient has complied with earlier treatment, or if a cure was successful). Besides pharmacological and clinical data, it is indispensable also to have some current knowledge about the microorganism and infection one wishes to diagnose: What the probable causes are, what detection difficulties exist or if some fastidious or unique pathogen is a possible causal agent. What possible detection means may be the most suitable, given the most probable clinical evolution, and if perfectibility to other patients is possible. Culturing may be the best diagnostic means, or maybe some additional (faster, more precise) diagnostic instrument is available. One must know if bacterial resistance is probable or anticipated, and if any co-infection or co-morbidity exists or is probable. Any preparing of the patient shall be pre- oriented and performed, for optimal results.

All those interrogations must be the object of the clinician´s consideration before ordering or writing and any special requests to the laboratory should. Answering them is paramount for the correct ordering, selection of exams and medical order.

The times of calligraphic hermeticism are long over: orders are to be presented either typed or in digital form, or else in clear writing; correct, explicit identification of the patient —as well as of the ordering physician — is indispensable.

Some examples of pre-analytical errors in the Microbiology Lab include [8]: incomplete requisitions, inefficient haemoculture collection, unacceptable specimens, due to failures related to identification, transport conditions, too long specimen transport times, etc.

Identification of the patient. Use of barcode identification and systems for the identification of the patient, sample and all materials can enhance security, but it is best if employed at all steps [1,2,13]. Meanwhile, the first contact of the first professional with the patient is what ultimately grants effectivity: if that is failed, all procedures will result inadequate. What is then fundamental before the preparation and collection of any laboratory exam is the patient's identification: Each patient must be unequivocally identified with a standard procedure, involving compulsory checking of some

ID document provided with the patient, with photo. For internal patients, unconscious or comatose individuals in an Emergency Room, etc., the first action of the collector must be to check for some identification, such as an ID bracelet, the immediate patient caregiver, a visiting parent or friend, etc. At least two information must anchor each sample: complete name, name of mother, ID number, unique personal records number at the hospital etc. If it is impossible to identify the patient permanently, some agreement as to a temporary id should mate (Such as: "the John Doe with a red shirt and blue eyes", etc.). A definitive ID should be established as soon as possible and linked to the initial one, such as to allow the logical connection of all results. It is unacceptable to proceed to any sampling or examination without a previous id since identification can be a significant source of error to all derived exams (both for the patient and potentially for others who might inadvertently present some similarity to him).

Preparation of the patient

Some tasks require specific measures from the patient [1,2]. In the case of a sputum sample for Bacterium as tuberculosis, the patient should know the procedure beforehand, and be aware that the best sample is taken at morning and in the well-hydrated state, and that a particular process may be necessary if his lungs are not abundantly productive at that moment. If the culture of a given wound is needed, he must know that, for at least 12 H beforehand, he shall not use any antimicrobial ointment, alcohol prep or other substance than water and saline solution, that may reduce or interfere with the recuperation of pathogens. On the other side, the clinician must know or be informed whenever a deep wound sampling is insufficient, or if a biopsy of the lesion should bear the best results. He must be aware of the bacterial flora related to the anatomical site of the collection—both about the potential pathogenic agents and to possibilities of its active recovery; whenever a specific anaerobe is a probable co-infector, that means the laboratory must adopt particular procedures. An unusual pathogen such as Neisseria gonorrhoea may be suspected, and that implies special procedures to enhance the recovery of that fastidious bacterium; or a cell smear may be needed to search for chlamydial inclusions. The clinical picture of the patient may have evolved in a way as to result in a different clinical manifestation that implies in the adaptation of the sampling. An alternative detection method— such as immunologic or molecular one – may be available and better indicated.

Sampling

Once decided for the specific exam and with the patient prepared, it is time to perform the collection. The professional who performs that must be aware of the best sampling procedure [1,2]. If a strep throat prep is needed the swab must not touch the oral anatomic elements other than the tonsils. A knee or abscess puncture requires an adequately trained collector, in a specially designated room, with adequate disinfection of the puncture site. When collecting a blood culture, one must decide how many samples (one, two or more) are necessary, and at what moment (before the estimated time of the fever or after it, or else all at different times [14]; clinical condition may require collecting all samples at the same time or grant the collection of specific anaerobe flasks [14]. Fungal or bacterial culture flasks may be needed. Due to the patient's clinical picture, he may not tolerate the accumulation of too large a blood sample. Other molecular tests, less blood-consuming, may be available.

Transporting. If the collection did not happen at the central lab, the sample transportation must follow to defined quality standards. Those must consider some characteristics of the sample, patient and microorganisms. Transport errors may be apparent as identification errors, preparation errors, or even the impossibility to recover or loss of a given sample [2].

Most bacterial samples can reach the lab in neutral media such as Cary-Blair's, provided the transporting do not take longer than two hours. However, if some slow-growing pathogen is suspected, a different media should be used; for some of those, the sample should stay at room temperature (20 - 24ºC) rather than refrigerated (2 - 8 C). If an anaerobe is a potential co-infect or, the correct technique includes the introduction of the obtained sample (either swab or biopsy) to a specific media, in a tight-sealed bag that carries anaerobic-generator chemistry; thus, anaerobic atmosphere must be soon generated, and the bag then very tightly sealed and kept so until processing in the central lab [15,16].

Urine and faeces for culture are generally sent in refrigerated state and shall reach the processing laboratory in about two hours.

Material for fungal identification and culture can be collected and transported in closed Petri-dishes or other large mouth bottles, securely enclosed and protected from humidity, light and temperatures above 35ºC.

Large-mouth bottles are also suitable for the transportation of sperm for culture and other fluids. Small flasks are adequate for Cerebral-Spinal Fluids (C SF). We must be attentive, though, to the fact that the collection of some fluids ordinarily sterile in the healthy man should be collected only after proper skin disinfection and always using hygienic materials (needles, syringes, and flasks). It is imperative for noble fluids such as C SF to receive suitable priority treatment and immediate transport to the laboratory for analysis.

In the example of CSF, it is habitual to collect it by dripping into several sequentially numbered small flasks. That serves for two purposes: on the one hand, all blood that may result from accidental vascular puncture tends to stay in the first and second flasks; on the other hand, the last container tends to be the least contaminated by external microorganisms [14]. However, that effect is in-existent when the collecting professional for some reason opts to aspire all the SCF sample with a syringe, and only then fill the flasks—rather than let it drip. In that case, equal labelling of all containers is mandatory, and both lab and physician must know the content in all flasks is homogeneous. For viral identification, SCF should be immediately stored at 4ºc, while for other pathogens it may be stored at 24/C [14].

When admitting samples into the processing lab, it is paramount to re-check and keep the identification of the sample. The ID should have at least two anchors, e.g., the complete name of the patient, mother's name, patient's unique ID, sample's unique identification. For the resampled items, all the sampling and resampling procedures must be traceable to the original.

All laboratories should keep an operational procedure clearly stating the criteria for acceptance and rejection of samples [1]. One should report any rejected samples to the administration of the lab, who shall establish any required correcting procedure. Refusing a sample should be carefully considered, because that may result in recalling the patient, or even worse, in loosing or compromising a potentially important exam, e.g. because the disease has progressed, or the patient is already on antimicrobials. Accepting any sample means the person who receives it is co-responsible to the result. Therefore, one must extensively review and check all acceptance criteria before the next steps. At that point, a good option is to use an aiding checklist. Partial acceptance means that a sample may be suitable for some procedures and determinations, but not for others—or else some of the analysis must require special consideration or some describing observation at the final report. Documentation of any of the absent criteria of acceptance should be clear, as those imply on alteration of any of the following steps. At the final point, when delivering results and diagnostics, a suitable remark must be made in the respective reports.

Any accepted sample shall be ready for the next steps

Some procedures include resampling (e.g., a given urine sample must be subsampled, with a sterile technique, into a fraction for standard urinalysis and a fraction for culture; the same may occur with a stool specimen – for routine coprology and stool culture; or a biopsy sample – for microbiologic culture and microscopy and staining at a Surgical Pathology. A semen sample for both routine sperm count and culture must remain in the original flask for about 30 minutes, at room temperature, in a clean environment, for the required analysis of liquefaction time; only then homogenisation follows, and the appropriate sample goes to the microbiology lab. When receiving a Synovial fluid flask, one must decide among using the vial collected with anticoagulant (may interfere, but also countermeasures can be taken) or the one without anticoagulant (there is the possibility of some agents remaining retained in a clot). When receiving CSF for analysis, the first procedures to be taken (if indicated) are generally those referring to fastidious bacteria such as Mycobacteria or Neisseria. The Microbiology lab shall be the first to open – in an appropriate sterile or clean environment – any samples for microbiological analysis; only after that may that sample be relayed to other sectors, for the less critical and less sterility-demanding procedures. However, some particular methodologies may be accessory. If you do not have fast and cheap molecular techniques for mycobacteria, letting a small sample of one of the flasks stand untouched for a few hours may reveal Mya's reticule, a thin spidery reticulate which is highly suggestive of M. tuberculosis.

The analysis is a complicated procedure that can be divided and subdivided in the required tasks [1]. The laboratory must document each process, and the decisions involved in that. Every laboratory is obliged to perform routine procedures of Internal Quality control and External Quality Control (IQC and EQC).

Some analytic errors on the microbiology lab are [8]: Internal and External Control failures; excessive frequency of failure of incubators and automators; Misinterpretations of results, or

equivocal results presented by automators. What quality control personnel should watch for is: Equipment reliability and correct installation; Reagent stability, integrity and efficiency; adequate temperature control; reliability, repetitiveness and accuracy of results; proper sampling and subsampling; proper recording of Automator's results and history keeping at automators.

Using IQC, the laboratories perform some controlled test(s) at each point or procedure, by using similar materials to the clinic ones, but of known results. E. g., when performing a batch Gram stain of several slides, one must include at least one slide that contains both a known Gram-Negative and Gram-positive control strain. When executing a Ziehl-Neelsen stain, controls consist of a known positive and a negative slide processed in that batch: the microbiologist looks at all the slides. Should the controls give results that are different from expected, the whole batch cannot be validated.

The use of ECQ enables further control of the procedures. For that, laboratories receive unknown materials from a third party. All laboratories (locally, nationally, or internationally) must individually and anonymously process those materials in a given schedule as if they were the ordinary clinical materials. Upon receiving of all results by the ECQ provider, that last one shall make a compilation and a statistical analysis of all achievements. After that, they routinely send a personalised, confidential report to each participant, classifying each analysis as either acceptable or not. Each lab gets its results, plus general anonymised statistics. That enables both self-evaluation and evaluation by the ECQ provider. As a rule of thumb, results inferior to a set-point (e.g. less than 80% of a given type of exam's results are considered adequate), the laboratory has a poor performance for that item and shall avoid further executing of that given exam in clinical materials until cleared. The lab must then search and determine the reasons for inadequacy, and retake specific examination of the corresponding procedure until he presents satisfactory results in a re-evaluating—revalidating method.

Complementary to the use of EQC and IQC is the use of statistics, derived from the analysis of those data and of any non-conformities that can be found and registered. The sequential evaluation of those statistics shall orient the administrators, as well as all participants, as to the most critical subjects at whom one must intervene. A number of those indicators have been proposed, and specific ones can be created by the analysts of the lab [2,11,17-26].

Some tasks inside the Microbiology with corresponding potentials of error are

Gram Staining, Ziehl-Neelsen or other stains

Stains are too old or outside validity;

Stains contaminated with microorganisms or other non-related chemicals; micro-crystals present on staining fluids (due to evaporation and concentration), that interfere with microscopy and difficult interpretation; Inadequate (amber or opaque) flask, temperature or light storage conditions; Other reagents such as alcohol and ether-alcohol are not of sufficient purity; Performing the staining procedure in disaccord to suitable technique (different stains, times, sequences, amounts etc.); Some unclean slides: tainted with inorganic powders from original packaging, grassy material from operator's hands, powder from gloves, lush materials in the sample, etc. – either during or after making of the smears, or during swabbing; The microscope is not suitable. Concordance between 2 or more participants can be determined by kappa statistics [12].

Plating

Inadequate preservation of sample with bacterial overgrowth; Inadequate preparing of the suspension for plating; Plates or other materials were not appropriately sterile; Streaking technique or plating technique was not appropriate to the sample or medical condition; The environment where the procedures were performed was not adequately clean or sterile; Inadequate subsampling of an item, or failure of streaking into the correct culturing media; a technologist or other professional fails to identify a sample or sub-sample correctly.

Incubating

Inadequate control of An incubator; Or a power peak occurred, and a bacterial incubator, haematology culture incubator, or Automator of identification/antibiogram analyser reached unstable or inadequate temperatures.

It is indispensable to submit to regularly all equipment incubating cultures to temperature and humidity control. If possible, with no-breaks, to resist electricity accidents; the latest machines come with continuous monitoring protocols, with local or even internet-based alarms whenever equipment reaches extreme conditions.

Fungal culture was subjected to heat above 35ºC. Alternatively, bacterial incubation reached more than 38 - 40ºC

Automation

Most laboratories nowadays adopt some automation procedures. There is a variety of automation, enabling simultaneous incubating blood culture flasks and detecting growth, thus alarming the technician when it is time to subsample;

Different equipment allows either Invasively or non-invasively subsampling of blood culture flasks (to better keep the original characteristics of the original sample in need of counter-proofing);

Regular plate incubators; partially automated (including inside incubation) determination of species and bacterial resistance etc. most automation has a system of the liberation of results in informatic form, either before or directly at the report. Some of them also include the possibility of automating the inclusion of specific observation about microorganisms, their detection, limitations of the procedure, clinical data related to the sample and microbial agent. However, all those data must be checked and reviewed by an experienced microbiologist. Also, we must consider that automation must have as input the adequate material, i.e., pure bacterial cultures. All the output and diagnostic data show inconsistencies and wrong diagnosis after any inadequate input to the analyser. Also, any microbiology analyser or system has limitations of its own. Most identifiers and analysers have limited profiles or set profiles. For some specific bacteria (such as anaerobes and fastidious), other procedures are necessary; for Antimicrobial Sensibility Evaluation, multi-resistant bacteria may require additional testing, such as disk diffusion or antibiotics absent from the analysis set, e-tests for the precise determination of Minimum Inhibitory Concentration (MIC), even other identification tasks such as the camp test. According to the current laboratory regulators (Eucast and NCCLS), not all automated procedures are adequate for the liberation of a diagnostic result without some additional testing. One must not forget that all samples must be considered as infective and preventing measures such as quick cleaning any spilling of suspected fluids, or else apply preventive medication for use accidentally infected, is mandatory. What one often overlooks is always the cleaning of the environment, liberating particulate matters and keeping the settings of insects and small rodents.

The post-analytical phase starts with the review and interpretation of the automated results and the required standardised tests. It is not advisable to directly liberate all results of any Automator, before individual evaluation. That evaluation may remain as a digital phenomenon or written documentation, but traceability as to the original materials and to final results must always be available. In some instances, automated *in vitro* results have to be corrected according to clinical rues – e.g., inducible penicillinases must be reported as resistant even if they are sensible *in vitro*. Lab professionals must check all information from automation systems against previous procedures such as Gram stain. E.g., whenever one observes a given report shows a Gram-positive bacterium – here the corresponding Gram stain did not show it – one must re-examine both items before reaching a conclusion. Gram stain is not considered compulsory in some laboratory, but it can be a valuable help when it comes to quality evaluation.

Once that analysis is satisfactory, the procedure of storage consisting in taking a part of the sample, or of the derived materials, and store it for a given period anticipating a possibility of counter-proof. Requisitions for that kind of storage are not fully standardised and may differ from laboratory to laboratory, and from country to country. The basic principle of that storage is to conserve the best and more significant fraction of the procedure (from sample o subsampling to final identification report), along with to its traceability, so that in case of need the process can be reproduced to check the results. Some significant challenges in Microbiology are: the big amount of materials, deciding what materials to store, and what are the ideal storage conditions are.

Validation of the results starts at the previous review and interpretation tasks and means all data in the exam must be considered—from the current procedures, CQI and CQE data, other microbiological exams, and their mutual consistency (Hayes, 1996).

A Biochemist, or suitably highly trained professional, must perform that task. Many additional data come into play—e.g., clinical data from the patient, anatomic site and its bacterial flora, the most probable pathogens, the clinician's diagnostic hypothesis. Perhaps what we are facing is a new bacterium or newly determined bacterial species that can or not be associated with the disease of that particular patient.

The finding of certain *staphylococcus* species in haemoculture requires careful consideration;

Sometimes we face emerging pathogens (or new emerging multi-resistant strains).

One should recheck all inconsistent data, and in the possibility of some unique fact, or a rare bacterial strain, program to enter a suitable observation may into the final report.

Those procedures integrate both laboratory findings (analysing all available lab data for that patient. Including those come from Immunology, Biochemistry and other) and delivery of results or elaborating of the final report. The final report must fit into a standard, schematic, clearly interpretable summary. The use of specific graphics may facilitate the clinician's visualisation and direct him to the more relevant findings. Any observations should be concise as well as clearly stated.

A supervisor or specialist may be assigned to evaluate those results after final analysis. That can part of a final delivery procedure or a safeguard.

The final files must be available at the laboratory archives, in case a copy or counter-proof is ever needed. Those are better kept digitised, for faster access and to take less room. The final saved files must include all traceability information, from the doctor's order to the final report. If at any time a review or counter-proof is needed, all documentation must be accessible. There must be no overwriting of original documents, as those may be testimony or an error or near error that represented equivocal input for other sequential acts.

Our story ends when the final report reaches the doctor. After reading the final report, he shall evaluate all the relevant data as to those diagnostic instruments. By estimating the new Positive and negative values, he is now able to determine the current health status of the patient. He may reconsider his initial clinical question and diagnostic hypothesis estimate if the disease process has evolved and how, then what are the required interventions. After those interventions, the process restarts, as the doctor must either check the results or determine if the patient's condition was cured or grants new acts, or if additional data may be required. Eventually, he discharges the patient after cure.

Summary

Microbiology exams, like any laboratory exams, are not self-limited and inelastic events. They are convoluted procedures, performed in a stepwise and systematic way. Attention to detail and quality is paramount at every step. All participants, from the patient to laboratory to the clinicians, must be aware of the main determinants of the quality of the results. Evidence-Based Healthcare is necessary for the whole procedure.

Especially the clinician may consider what determines given results, how he is to interpret it and if he eventually needs support and enlightenment from the Laboratory. Comprehensive care for the patient requires an integrated communication between laboratory, patient and physician whenever some questioning as to the procedure arises.

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