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Clinical laboratories – production factories or specialized diagnostic centers

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ABSTRACT

Since a large proportion of medical decisions are based on laboratory results, clinical laboratories should meet the increasing demand of clinicians and their patients. Huge central laboratories may process over 10 million tests annually; they act as production factories, measuring emergency and routine tests with sufficient speed and accuracy. At the same time, they also serve as specialized diagnostic centers where well-trained experts analyze and interpret special test results. It is essential to improve and constantly monitor this complex laboratory service, by several methods. Sample transport by pneumatic tube system, use of an advanced laboratory information system and point-of-care testing may result in decreased total turnaround time. The optimization of test ordering may result in a faster and more cost-effective laboratory service. Autovalidation can save time for laboratory specialists, when the analysis of more complex results requires their attention. Small teams of experts responsible for special diagnostic work, and their interpretative reporting according to predetermined principles, may help to minimize subjectivity of these special reports. Although laboratory investigations have become so diversely developed in

the past decades, it is essential that the laboratory can provide accurate results relatively quickly, and that laboratory specialists can support the diagnosis and monitoring of patients by adequate interpretation of esoteric laboratory methods.

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INTRODUCTION

Since about 2/3rd of medical decisions are based on laboratory test results (1), it is obvious that clinical laboratories need to be organized in the best possible way to meet this demand. Optimizing, in the economic aspect, usually means fusing smaller units into larger ones to save costs, as well as trying to automate as much as possible. Undoubtedly, this has been an ongoing tendency for decades and has resulted in centralized, mega-laboratories that may process 15-20 million tests per year. There are two key concepts in these large laboratories: integration, where analytical instruments or groups of instruments are linked with pre- and post-analytical devices, and consolidation, where different analytical technologies or strategies are combined in one instrument or in a group of connected instruments. However, there is a logical limit to centralization, since no laboratory expert anticipates that a dozen 'ultra-mega-large' laboratories would be enough for a mid-size european country, or that these laboratories would be the best from the point of view of optimal patient care. Politicians and health economists, on the other hand, often tend to think differently, and, as they are unaware of the details of the laboratory professsion, such conceptions may actually prevail.

The majority of the laboratory tests are basic clinical chemistry, hematology, urinalysis and hemostasis screening tests. In many smaller laboratories this comprises the whole repertoire of the laboratory. There are two expectations from the patients and their caretaking doctors: the results should be accurate and they should be delivered fast. The laboratories are putting a lot of effort in the former by using internal and external controls, investigating interfering factors and linearity values, however laboratories are sometimes not paying enough attention to delivering the results on time. The timely delivery of laboratory results, however, is also very important. It may become unnoticed by the doctor if the laboratory is underestimating an enzyme activity by 10%, but the clinician probably does not accept if the same result is delayed by a few hours.

METHODS TO IMPROVE LABORATORY PERFORMANCE

The measuring clock of clinicians' satisfaction: turnaround time

Thus, each laboratory should monitor this key 'satisfaction factor' entitled turnaround time (TAT) and try to improve it as much as possible (2-5). One way for improvement is to modernize courier services in hospitals. The past years have proved that this is best achieved by automated transportation systems, the most widely used method being the pneumatic tube system. In these long tube systems that may reach a complete length of over 20 kilometers, numerous compressors are utilized that produce the pressure for independent circuits, which transport the capsules containing the laboratory samples. In the advanced systems, automated capsules are used, i.e. the capsule itself does not appear in the laboratory, but after its content is automatically unloaded, it returns to the station of origin by the aid of a radiofrequency tag that is attached to the surface of the capsule. Such systems can also optimize the travelling speed of the capsule as being faster when the capsule is empty and slower when it is carrying a sample

(6 m/s versus 3 m/s) (6). Another way to decrease TAT is to deploy laboratory testing to the actual site of patient care in the form of point of care testing (POCT). All POCT results, however, should be registered in the laboratory information system (LIS) and only results that are appropriately controlled, registered and validated should be used in patient care. (7)

Central laboratories usually have three types of assays based on TAT:

- *Emergency testing.* Here the complete 'from vein to brain' TAT should be below 60 minutes. In some cases, extra-urgent samples may need to be further prioritized, such as in the case of patients with ischaemic stroke waiting for thrombolysis.
- *Routine testing.* The TAT for routine test results today may be quite close to the emergency results, but a more realistic maximal routine TAT value is 3 hours. Nevertheless the median TAT for most the routine assays is around 80-90 minutes.
- Special *testing.* The TAT for these assays may be highly variable ranging from 2-20 working days. It can be assumed that no laboratory test should take more than 20 working days, as it would not be possible to effectively implement those slowly generated results into actual patient care.

The first two types of testing are usually part of the 'production factory' (8) while special testing occurs in specialized centers. A delicate balancing is required to devote sufficient resources from the laboratory to each of these test groups.

Ways to optimize test ordering

While we provide a medical service for the patients, whether we like it or not, with a large part of laboratory testing we implement a factory-type work flow, mostly for bulk tests described above (9). It may be assumed that, indeed, doctors often use too many diagnostic tests, and these tests are requested too frequently. This may be because they have erroneous expectations of the tests, are unaware of tests carried out previously, or are simply trying to be rigorous. Because these tests can be easily requested, it has been estimated that 8-30% of test requests may be superfluous (10). Thus, it is plausible that laboratory performance may also be improved by eliminating overtesting. This is, however, somewhat difficult to carry out optimally, and several techniques have been suggested to manage, or rather, to limit the ordering of test requests. One option is to allocate the whole laboratory budget to the requesters or to use a computerized clinical decision support system (CDSS) in medication as well as laboratory test ordering. Most other possibilities refer to tricks that the laboratory can do to prevent overtesting. These may include discouraging or not automatically fulfilling test requests, or creating explorative and reflective testing, such as beginning with a nonspecific, cost-effective but sensitive test, and then performing more targeted and usually more expensive tests only when the results of the initial screening tests are abnormal. A quite useful method could be to exert influence through setup of request forms, or to reduce the availiability of testing at certain times. A relatively low percentage of superfluous tests can also be eliminated by the laboratory through barring tests on predetermined principles of frequency filtering (11).

How to make the most of the laboratorians' time: autovalidate

One way to achieve meaningful organization is by automated evaluation of laboratory results for straightforward cases using autovalidation. If a laboratory is not using autovalidation in 2016, it is frustrating for the laboratory specialists, who are under constant pressure to devote their skills to checking the correctness of tens

of thousands of numerical values for 'simple cases', which may belong to any of the groups below:

i. each laboratory result is within the age specific reference range;

ii. only minor, clinically insignificant laboratory changes occur or

iii. many laboratory results are pathological, but all are similar to preceding values and are compatible with the diagnosis provided.

Tedious manual validation of simple cases by laboratory specialists carries the risk of serial mistakes, since after a while it is impossible to responsibly evaluate large quantities of data. Additionally, this laborious task takes the expert laboratorian's attention away from quality validation, where their time should be devoted to more complex cases.

In a large laboratory with a wide portfolio, the following simple rule may apply:

- Around 90% of the samples require 10% attention and
- The remaining 10% of the samples require 90% attention.

REPORTING AND INTERPRETATION OF SPECIAL LABORATORY RESULTS

Expert opinion of simple tests

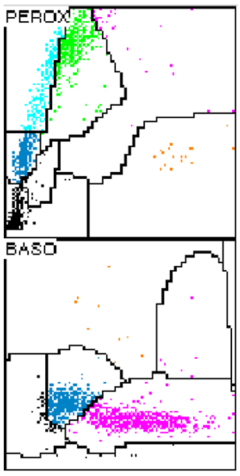
Now, what are those 'more complex' cases that require considerably more time than a handful of 'simple cases'?

If we just consider the basic laboratory portfolio, several complex cases could be mentioned. The automated hematology analyzer reports should be confirmed and validated, since falsely low neutrophil percentage may be reported with erroneously high monocyte numbers in cases with partial or complete myeloperoxidase (MPO) deficiency (12) if differential counts are based on volume and MPO activity (Figure 1A). In the case of unexpectedly high creatine kinase activity, further testing may be required to verify the presence of type I and type II macro-CK isoenzymes, an entity that results in falsely elevated CK-MB values in the immunoinhibition test

Figure 1 Production factory: cases when more attention is needed in the routine laboratory

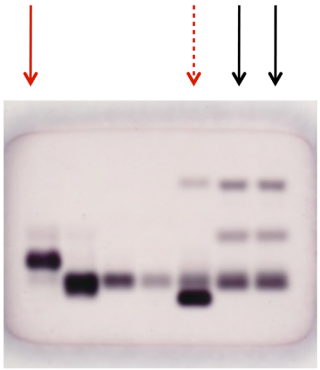
A

Types of white blood cells	Results on hematological analyzer using myeloperoxidase staining	Results on hematological analyzer using Coulter principle	Reference range
Neutrophil	**8.4%**	**62.5%**	43.0 – 76.0%
Lymphocyte	28.5%	29.4%	17.0 – 41.0%
Monocyte	**48.1%**	**5.6%**	3.4 – 9.0%
Eosinophil	1.8%	2.4%	0.0 – 5.0%
Basophil	0.3%	0.1%	0.0 – 1.5%
LUC	**12.9%**	-	
MPXI	-40,7	-	-10.0 - 10.0

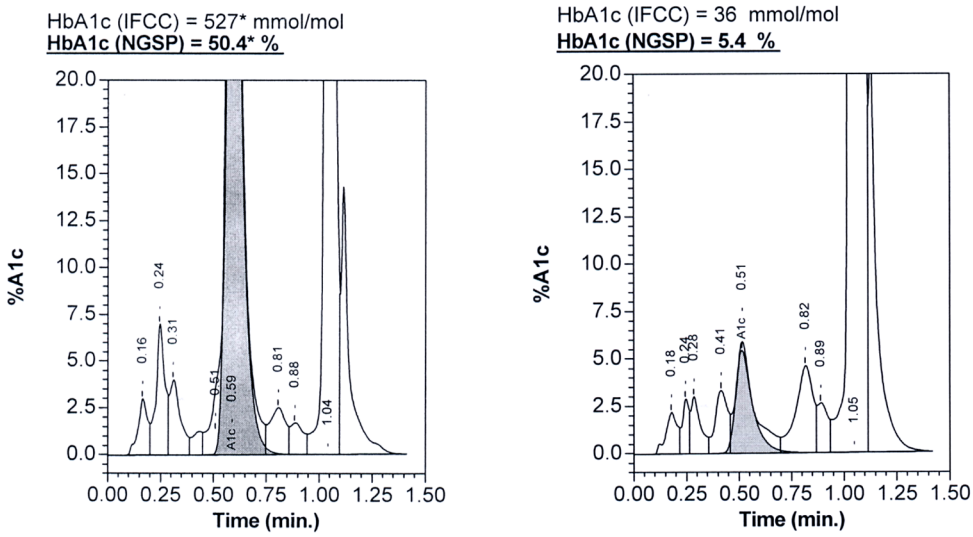


B

CK isoenzymes	Patient 1	Patient 2	Reference range
CK-MM	5.7%	20.7%	92 – 100%
CK-MB	3.6%	0.9%	< 6%
CK-BB	0.1%	3.7%	< 2%
Macro CK 1.	**90.6%**	0.0%	
Macro CK 2.	0.0%	**74.7%**	
Atypical isoenzyme	0.0%	0.0%	



C



(A) Myeloperoxidase deficiency results in decreased ratio of neutrophiles, and elevated ratio of monocytes and large unstained cells (LUC) when the sample is measured on hematological analyzer using myeloperoxidase staining.

(B) Macro CK results in high CK activity and disturbs the measurement of CK-MB activity using immunoinhibition method. On the CK electropherogram either macro CK 1 (red continuous arrow; patient 1), or macro CK 2 (red dotted arrow; patient 2) are shown compared to control (black arrows).

(C) Extremely elevated hemoglobin A1c concentrations can be measured by HPLC in the presence of some rare hemoglobin variants (e.g. Sherwood forest hemoglobin variant).

(Figure 1B). Similarly, a clinically silent hemoglobin variant, like the rare Hemoglobin Sherwood, can cause an extremely high value in the automated HPLC testing for glycated hemoglobin, where the diagnosis is provided by mutation analysis (Figure 1C). In addition to such cases several other areas exist that require interpretative reports (13) that has been shown to contribute to physician satisfaction (14). Aside from such cases, most of the quality time for general

routine analysis is devoted to microscopic investigations of peripheral blood or cerebrospinal fluid samples.

Expert opinion of special tests

Another area of interpretative reporting is when samples are sent for more esoteric tests, and in many cases no test requests are indicated, rather, a hypothesized diagnosis need to be confirmed or rejected.

These types of investigations mostly, but not exclusively, involve flow cytometric analysis of peripheral blood or bone marrow, cytogenetic analysis for G-banding or FISH, autoantibody pattern description, dynamic endocrine tests and special hemostasis assays for bleeding diathesis or thrombosis. Many of the nucleic acid-based tests can now be easily set up, but in some cases whole-genome sequencing and the interpretation of rare mutations may take many hours, or even days of qualified work from the laboratory specialists to delineate the diagnosis. Many of these techniques also require months or years of experience/training to gain sufficient expertise. Morphological skills are essential to evaluate pathological peripheral blood, bone-marrow or cerebrospinal fluid samples, or to describe autoantibody staining patterns. However, sometimes these skills become additional to other specialized skills, such as the ability to confidently read DNA sequencing curves, operate the software for flow cytometric dot plot analysis, or learn the details of a karyotyping software operation. It is also imperative to sustain the TAT concept in the case of these special tests. This means such a service cannot rely on a single expert, thus a minimum team of two people should handle the reports in each of these subspecialities. The best scenario, however, is a team of about three experts who take turns writing the reports while sticking to predetermined principles of data reporting to minimize subjectivity of these special reports. In our Department, the Divisons that exert the highest time-demand for special diagnostic work are summarized in Table 1. In all of these Divisions, a minimum of 3-5 specialists take turns reporting, and in some areas, two people are required for one type of subspeciality for parallel reporting.

These reports have a generally accepted format and the result sheet should include considerably more data than a general chemistry assay.

A typical request of clinical flow cytometry for the investigation of hematological malignancies should include the followings (15):

- demographic identification of patient;
- identification of the hospital or division sending the sample;

Table 1 Special diagnostic work with the highest time - demand in the Department of Laboratory Medicine at the University of Debrecen

Special divisions with the highest time-demand of diagnostic work	Special diagnostic work (hours/week)	Annual interpretative reports (and its ratio of reports of division)	Turnaround time (working days)
Flow cytometry	70-80	3 000 (67%)	3
Molecular genetics	50-60	2 400 (33%)	12-20
Laboratory immunology	50-60	1 200 (5%)	10-20

- type of specimen (bone marrow aspirate, peripheral blood, other biological fluids);
- timing of observation (first sample or follow-up);
- diagnostic hypothesis of the sender.

When reporting the results of the flow cytometric analysis, the following elements are required (15):

- list of antigens and type of immunofluorescence analysis;
- absolute number of cells in the sample;
- quality of the sample, in terms of viability;
- general description of the gating procedure;
- immunophenotype of blast cells;
- description of cells surrounding blasts;
- diagnostic conclusions.

In special cases, other parameters may be required, like the definition of an antigen panel for the detection of minimal residual disease. In addition, a representative dot plot is also part of the interpretative report. These attributes minimize the subjectivity of the special reports. Nevertheless, there are several flow cytometric analyses that do not require interpretative reporting. This usually depends on the question raised, when reporting of a sheer number is sufficient, like in the case of CD34 positive cell count, or when a qualitative answer is required, like in the flow cytometric heparin-induced thrombocytopenia assay.

Another example is genetic test reports that undoubtedly carry a serious clinical implication for prediction of susceptibility to disease, patient diagnosis, prognosis, counselling, treatment or family planning. Therefore, such laboratory reports should provide a clear, concise, accurate, fully interpretative and authoritative answer to the clinical question (16).

These reports should include a clearly structured format, comprised of the following information:

- administrative;
- patient and sample identification;
- restatement of the clinical question;
- specification of genetic tests used;
- results;
- interpretation of results.

Upon interpreting the results, the expert draws a conclusion that should contain any of the five subsequent possibilities:

- normal finding(s);
- non-specific finding(s) without clinical relevance;
- incidental finding(s) with possible clinical relevance;
- finding(s) of uncertain significance;
- pathognomonic (disease-specific, pathological) finding(s).

When a new diagnosis is made based on these reports, it is appropriate to state specifically that the result has 'potentially important implications for other family members'. Depending on the context, it may be appropriate to explicitly mention the recommendation to test the partner, the possibility of cascade screening tests in relatives, and the possibility of prenatal diagnosis or preimplantation genetic diagnosis. Genetic testing is unique in the respect that when appropriate, the risk for future offspring should be calculated and provided.

Several other areas of laboratory medicine exist where interpretative reports are required. One such area is autoantibody testing. In these studies, two or more methods are frequently used to identify an antibody marker and sometimes the results disagree. When this happens, an interpretation is always required, specifying the

diagnostic accuracy of the tests. Similar to the previously described genetic tests, some autoantibodies that are not requested, and consequently not expected, may be identified by chance. Such cases should only be interpreted when these antibodies have a significant clinical correlation (17). Examples from our laboratory are provided for interpretative flow cytometric, genetic and autoantibody reports in Figure 2.

Since laboratory tests are usually requested by well-trained clinicians who are aware of the diagnostic, prognostic and monitoring value of the results, the over-interpretation of self-explanatory numerical tests can be useless and

Figure 2 **Specialized diagnostic centers: interpretation of special laboratory results**

A

Sample type	Bone marrow
Panels	Panel of acute leukaemias, de novo T-ALL, AML
CD markers	CD2,CD3,CD4,CD5,CD7,CD8,CD10,CD13,CD14,CD15,CD16,CD19,CD1A, CD30,CD33,CD34,CD38,CD45,CD56,CD64,CD71,CD99,CD117,CD11b,CD123, CD300e,cy3,cyFXIII,cyMPO,cyTdT,HLA-DR,syto16
Interpretation	Nucleated cell count in the bone marrow sample was 110 G/L. Besides 2% mature lymphocytes, 2% erythroid precursors and 4% mature myeloid cells 87% blasts were detected based on CD45 expression and side scatter characteristics. Blast population is not homogenous, blasts belong to two different cell lines, 15% of the bone marrow cells are T lymphoblasts characterized by an immature immunophenotype: CD7+/CD99+/nTdT+/CD34+/CD10+/cyCD3+ and CD45-/CD3-/CD2-/CD5-/CD4-/CD8- while 72% of the bone marrow cells are pathological monocytes with CD64+/CD33++/cyFXIII+/CD4+/cyMPO+/CD15dim and CD14-/CD11b-/HLADR+-/CD13-/CD34-/CD117- immunophenotype. Based on the WHO 2008 classification of acute leukemias the formerly bilinear leukemias are now classified as MPAL, in this case consisting of myeloid/monocytic blasts and T lymphoblasts. **Summary: MPAL, with 72% myelo-monocytic blasts and 15% T lymphoblasts in the bone marrow aspirate.**

B

Sample type: Bone Marrow
Referral Indication: MDS? Neutropenia and low platelets
Karyotype: 46,XY,der(7)t(1;7)(q10;p10)[15]
Interpretation: Analysis of fifteen metaphases from the bone marrow showed an abnormal clone containing a translocation between the long arm of chromosome 1 and the short arm of chromosome 7 with breakpoints at 1q10 and 7p10. This translocation results in the deletion of the whole long arm of chromosome 7 and trisomy of the long arm of chromosome 1. The result is consistent with the referral indication and confirm the diagnosis of MDS. MDS associated der(7)t(1;7)(q10;p10) occurs as a sole abnormality in the majority of cases. According to the literature, this unbalanced translocation defines a unique clinicopathological subgroup of myeloid neoplasms. The der(7)t(1;7)-positive MDS cases show lower blast counts and higher hemoglobin concentrations at diagnosis and slower progression to acute myeloid leukemia than other -7/7q- cases.

C

Test	Result	
Liver specific autoantibodies (immunoblot)		
AMA M2 antibody	negative	
Anti-Sp-100 antibody	positive	!
Anti-LKM1 antibody	negative	
Anti-GP 210 antibody	negative	
Anti-LC1 antibody	negative	
Anti-SLA antibody	negative	
Autoantibodies on rat tissues (rat LKS IIFA)		
Anti-smooth muscleantibody	negative	
Anti-LKM antibody	negative	
Anti-gastric parietal cellantibody	negative	
Anti-mitochondrion antibody	negative	

Test	Result		Reference range	Unit
Anti-cellular antibodies (HEp-2 cell IIFA)				
ANA pattern 1	multiple nuclear dots	!		
ANA titer 1	> 1:5120	!	< 1:160	titer
ANA pattern 2	speckled	!		
ANA titer 2	1:5120	!	< 1:160	titer
Anti-cytoplasmic pattern	negative			
ANCA IIFA				
C-ANCA	negative			
P-ANCA	negative			
Atypical ANCA	negative			
Anti-MPO antibody (ELISA)	<5,0		<5	U/mL
Anti-PR3 antibody (ELISA)	<10,0		<10	U/mL

Interpretation:
Antinuclear antibodies of multiple nuclear dots and speckled patterns are detectable in high titer. MND pattern is caused by anti-Sp-100 antibody which is highly specific for primary biliary cirrhosis. However it may be present in other diseases (such as rheumatoid arthritis, systemic lupus erythematosus, systemic sclerosis, Sjögren syndrome) and may precede disease symptoms by years. To elucidate the antigen specificity of the speckled ANA component anti-ENA antibody determination is recommended.

(A) An interpretative flow cytometric report of a patient with mixed phenotype acute leukaemia
(B) karyotype determination of a patient with myelodysplastic syndrome
(C) the interpretation of autoantibody pattern of a patient with primary biliary cirrhosis are shown

harmful. However, laboratory investigations have become so diversely developed in the past decades that in the aforementioned cases, as well as in case of many other special tests, it is essential that the laboratory specialist provides a meaningful interpretation to the laboratory findings.

CONCLUSION

A clinical laboratory should be organized in a way so that the clinical pathologist can utilize most of his/her trained skills in evaluating results of specialized diagnostic areas and in interpreting laboratory reports for the physicians. This can be best achieved by introducing automated evaluation in the form of autovalidation in several routine laboratory fields in case of numerous samples that do not require direct medical surveillance. All these measures would facilitate that the laboratorian will become an indispensable part of the medical team.

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