Extended Neonatal Metabolic Screening by Tandem Mass Spectrometry: Models and Simulation of Alternative Management Solutions

Arturo Liguori
Epidemiology and Community Medicine Unit,
Department of Paediatrics - University of Padova
Via Pietro Donà 11, 35129 Padova, Italy
e-mail: epi@pediatria.unipd.it
phone: +39-049-8215700, fax: +39-049-8215700

Giorgio Romanin-Jacur
Department of Management and Engineering
University of Padova
Stradella San Nicola 3, 36100 Vicenza, Italy
e-mail: romjac@dei.unipd.it
phone: +39-335-6072747, fax: +39-0444-998888

Abstract- Neonatal metabolic screening aims at identifying newborns with severe metabolic pathologies in order to promote appropriate interventions to avoid or to improve adverse outcomes. Tandem Mass Spectrometry permits, from a blood drop, collected on a blotting paper by a puncture on the heel, to measure a lot of metabolites according to their mass; this method can identify more than 30 metabolites, each of which is a potential marker of a hereditary metabolic disease. The large amount of available information and the difficulty in correctly interpreting them in a short time, compatible with the exigencies of newborns, imposes to find an optimal management of structures devoted to perform the related tests. In the paper four different solutions, based on different utilizations of a cluster of two or more test structures, are examined and evaluated. A simulation model, coded in language Arena, has been built to get numerical results; such a model may be usefully employed to compare the effects of different solutions for an actual situation and to give a correct dimensioning to the chosen solution.

Keywords- Metabolic hereditary diseases, neonatal screening, tandem mass spectrometry, test structure cluster, conceptual model, simulation.

1 INTRODUCTION

The screening is the application of a test to all subjects of a population with the scope of identifying a disease at the moment when it is asymptomatic. A screening test has not the significance of a diagnostic test: it identifies a person who appears to be sound but probably suffers from a disease among people who do not; people with either positive or suspicious result shall be sent to the doctor for diagnosis and necessary therapy [10, 14, 22].
The idea of an early disease diagnosis and treatment is simple; conversely the path, which brings on one side to care people for a previously undiagnosed disease and on the other side not to damage those who do not need any treatment, is not simple. Then in [37] leading criteria were fixed to identify pathologies for which a screening plan is appropriate; such criteria were repeatedly updated and are synthetized in [1, 10, 14, 16, 30].
The scope of neonatal screening lays in identifying newborns with severe pathologies in order to promote appropriate interventions to avoid or to improve adverse outcomes [35].
Biochemical mass test on newborns began in 1960 with the adoption of a screening for phenylketonuria, a rare congenital metabolism error which, if not treated, leads to a severe mental retardation. Gutrie in 1960 developed a methodology to measure phenylalanine concentration in blood; this test required a few blood drops taken from the heel and soaked into a blotting paper now known as Gutrie card [20]; moreover it has the characteristic of being quickly effected on a large sample number. Such a test first became compulsory in Massachusetts in 1963, but soon in many states neonatal screening test plans took place. Note that the same sample may be used for many tests, so that other pathologies were introduced in the neonatal screen plans.
In the 90's the development of tandem mass spectrometry brought a great change to neonatal screening, as this method permits identification of a large number of metabolites from the same sample of a few blood drops on the blotting paper, so that the screening for 30-40 metabolic pathologies is possible. Pilot plans developed in Australia and New England studied tandem mass screening effectiveness and revealed a higher identification capacity if compared with clinical diagnoses [34, 36, 38]; moreover results showed the advantage of better prognosis of identified and precociously treated patients.
Neonatal screening plans with tandem mass technology were developed in Australia, Canada, Qatar, Taiwan, Turkey and in the majority of U.S.A. [6, 11, 12, 15]. In Europe 24 states activated such plans, either applied to the whole country or limited to some regions [2, 3, 9, 13, 19, 23, 25, 26, 27]. The set of screened metabolic diseases is different among the various states.
The extended neonatal screening performed by the technique of Tandem Mass Spectrometry (MS/MS) represents an approach of absolute importance to hereditary metabolic diseases' screening [4, 5, 18, 28, 29, 32, 33]. As already mentioned above, this methods permits, from a very small volume of biological material, to measure a lot of substances of intermediate metabolism (metabolites), which form during chemical reactions transforming nutrients (like proteins and fats) into energy. Metabolites can be measured in a blood drop, collected on a blotting paper (Guthrie paper) by a puncture on the heel, by means of tandem mass spectrometry which identifies them according to their mass, which is a physical characteristic of every metabolite. This method can

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identify more than 30 metabolites, each of which is a potential marker of a hereditary metabolic disease [7, 8].
MS/MS method produces large available information; on the other side such information shall be correctly and uniquely interpreted in a quick time, or anyway a time compatible with newborn exigencies (possible therapy shall be started within a time between one week and four weeks according to the specific disease); that imposes the necessity of identifying an efficient methodology for the management and organization of tests performed within neonatal screening [31]. Moreover we have to state very clearly (also on the basis of experiences reported in the literature or performed in international centres) the modes of behaviour in the case the screening yields positive results. Eventually the necessity of reducing the recall rate, i.e., the amount of newborns who are addressed to the clinical diagnosis and possible therapy, is due to the scopes of reducing both the workload of diagnosis and care structures and the stress of newborn families. Therefore it is essential to have available predictive models able to evaluate system operations in the phases of model adjustment and maintenance, related to modification of decisional trigger values for single pathologies, obtained by considering follow-ups to screening results (number of positives, false positives and negatives). A second test on the original screening sample with positive screening result, performed to specify whether the infant is affected before addressing the infant to the clinical diagnosis and possible therapy may reduce the number of false positives: such operation is known as second-tier test [17, 21]. This should be considered as part of a unique screening strategy. The versatility of MS/MS makes it possible to utilize this technology for 2-tier screening as well as for primary newborn screening, after suitable machine tuning.
Necessary equipment and structure costs, together with management and dedicated personnel costs are to be seriously considered related to expected results.
A feasible solution to many management and organizational problems is offered by the chance of using two or more machines in cluster, either with different roles or in concert to obtain a unified goal.
The paper aims at comparing different solution to utilize resources already present on the territory, in order to suggest an optimal organizational mode for a cluster of structures potentially insisting on the same catchment area. Merits and defects of every configuration are evaluated with the following objectives:
- to better manage every (positive or negative, true or false) sample follow-up, by setting a predetermined path for every possible outcome;
- to reduce the number of false negative results (and consequently the number of newborns who present pathologies but are not recognized by the system) by possibly setting an analysis path including differentiated tests characterized by high sensitivity and high specificity (2-tier);
- to rationalize the overall test method by organizing the structures so to permit, in times compatible with newborn exigencies, the reduction of the number of newborns addressed to clinical diagnosis;
- to reduce times necessary to taken samples to cross the whole diagnostic system;
- rationalize equipments and structures utilization by considering the overall number of treated samples;
- rationalize territorial resources and teams utilization so to avoid useless overlapping and to obtain an effective and unique coordination.
Moreover the paper presents both conceptual and simulation models able to compare different distributions of resources and tasks to two or more centres serving the same catchment area.

2 BASIC ASSUMPTIONS

While configuring the suggested models some basic elements of the literature were considered.

2.1 Numerical dimension of catchment area

The correct operation of a screening system depends on the knowledge of markers within people who does not suffer from diseases (reference values) and within people who suffer from diseases, on the correct setting (and successive tuning on the basis of feedback analysis) of the Cut-off to separate the two populations, on the definition of precise decision trigger values for any consequent action.
The applied solution shall permit to compute reference values on the basis of a sufficiently large population, by using the same diagnostic instrument, so to compare real results detected on the territory with reference values reported in the literature, with the scope of increasing the quality of extended neonatal screening).
In the configuration of many stand alone centres the typical dimension of 30,000 samples per year is not reached, while on the basis of international experiences reported in the literature a minimum number of 35,000 and an optimal one of at least 50,000 samples per year is suggested, justified by the following elements:
- plant and structure cost amortization (the break even is normally placed at 35,000 samples per year);
- rarity of some pathologies does not permit, particularly with small catchment area, to build up a sufficient experience for operators and above all to correctly set trigger values and cut off points for the population where the centre is placed, on the basis of analytical results after the screening phase;
- suboptimal dimensions of catchment areas of many projects make the screening system self-learning period particularly long and difficult [31].
Note that the dimension of catchment area for a single centre may reach values much larger than 50,000 samples per year, for instance in Europe in 2007 there were centres able to manage up to 77,000 samples per year [3].

2.2 Structure specialization

The configuration of an only screening cluster based on the specialization of two or more structures within it actually

permits to obtain a remarkable improvement in the quality of performed analyses. That mainly happens as, if correctly dimensioned and managed, a cluster can permit either a different setting of system machines, with no risk of increasing the number of false negatives, or the use of the same machine with frequent setting changes between high sensitivity and high specificity, without substantially affecting the sample crossing time.

3 COMPARISON AMONG CONCEPTUAL MODELS

3.1 Generic system

A generic system which would be applied in the case of an only centre, considered only as a reference, is represented by a block diagram in Figure 1, where the sample movement inside the system is clearly reported.

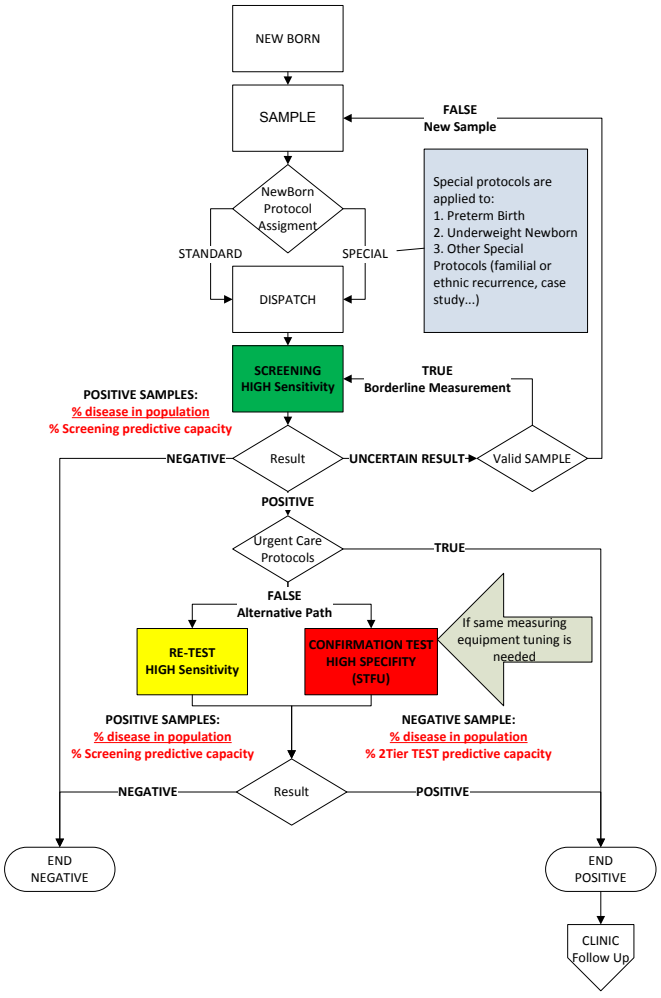

Figure 1. Generic test system.

The presented model describes the standard operation of a screening centre performing sample test and possible retest in case of positive result, either at high sensitivity or at high specificity (second tier) after a new machine tuning; the second confirmation test at high specificity is also called Short Term Follow Up (STFU).

The model can be simulated by using three parameters for every pathology:
- the pathology incidence on the population
- the predictive capacity of the specific test (true positives/true+false positives) observed in the screening phase, with measurement in condition of high sensitivity
- the predictive capacity of the specific test (true positives/true+false positives) observed in the screening phase, with measurement in condition of fist tier high sensitivity test and second tier high specificity test, after different machine tuning.

The four studied systems with two analysis centres are represented by block diagrams in Figures 2, 3, 4, 5, which represent four different cluster configurations of screening structures:

1. Cluster with geographical distribution of population on the specific competent centre, where structures perform only high sensitivity screening;
2. Cluster with geographical distribution of population on the specific competent centre, where one structure performs only high sensitivity screening and the other not only performs high sensitivity screening but is at disposition for second tier analysis at high specificity with different machine tuning;
3. Cluster with geographical distribution of population on the specific competent centre, where both structures perform not only high sensitivity screening but also second tier analysis at high specificity with different machine tuning;
4. Cluster with centres specialization and differentiation in terms of catchment areas and utilization of machines and structures.

3.2 Cluster with geographical population distribution, only high sensitivity screening

The first solution described in Figure 2 represents the most common current configuration: two or more centres, even close to one another for what concerns the catchment area, are utilized by simply allocating the population according to territorial competence, trying at most to get numerical balance. In this case we hypothesize that centres limit their activity to performing a high sensitivity analysis so to reduce at most the amount of false negatives and in the meantime to keep the sample crossing time restrained. In any case the retest is planned for those samples which in the first instance give positive result, but without adopting different tuning for the screening machine (in order to reduce the amount of false positives).

The advantages are:
- restraint of false negatives amount;
- high sample screening speed;

and the disadvantages are:
- high amount of false positives (and consequent address to clinical structure for diagnosis, possible therapy and follow-up planning), not restrained by a high specificity second tier test able to increase predictive capacity.

This method proves to be especially effective in the case the catchment area related to every centre is close to maximum analysis capacity of available machines and structures.

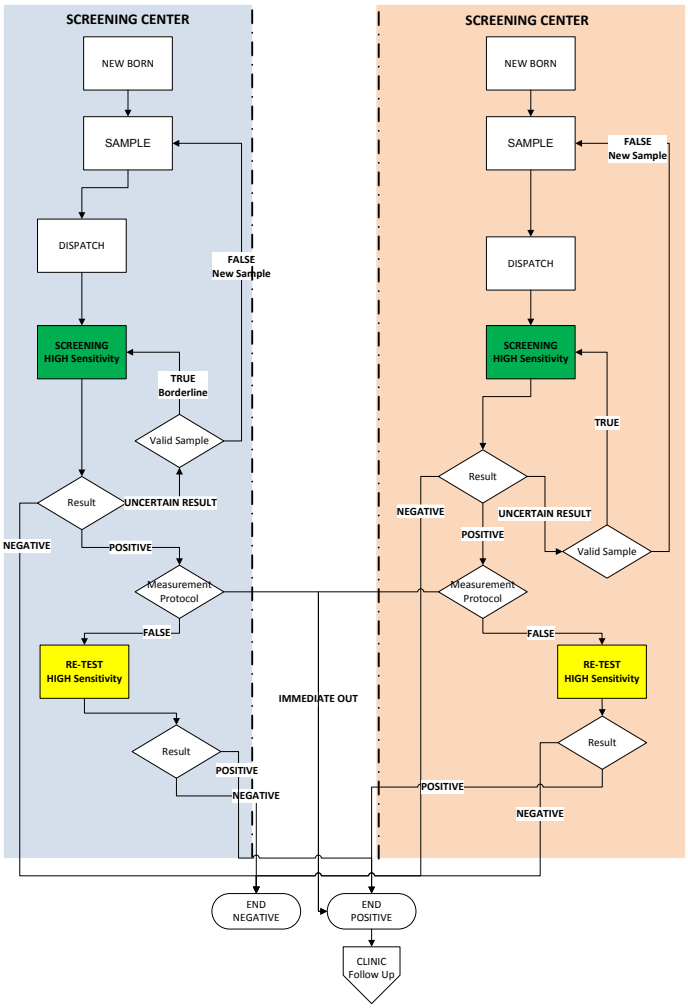

Figure 2. Cluster with geographical population distribution, only high sensitivity screening.

The block diagram in Figure 2 is divided into two parts which represent both the geographical placing and the different responsibilities of involved institutions.
The model operation is the following:
1. The sample is taken and sent to the competent centre;
2. A first high sensitivity screening is performed (to reduce the chance of a false negative);
3. In case of uncertain result the test is newly performed if the sample is good, otherwise a new sample is taken and the test repeated;
4. If the screening gives negative outcome, the sample goes out of the system, otherwise a retest is performed on the same machine with the same tuning;
5. In case of confirmation of positive outcome by the retest the newborn is addressed to the clinical centre for diagnosis, possible therapy and follow up.

3.3 Cluster with geographical distribution of population on the specific competent centre, where one structure performs high sensitivity screening and the other also performs high specificity retest.

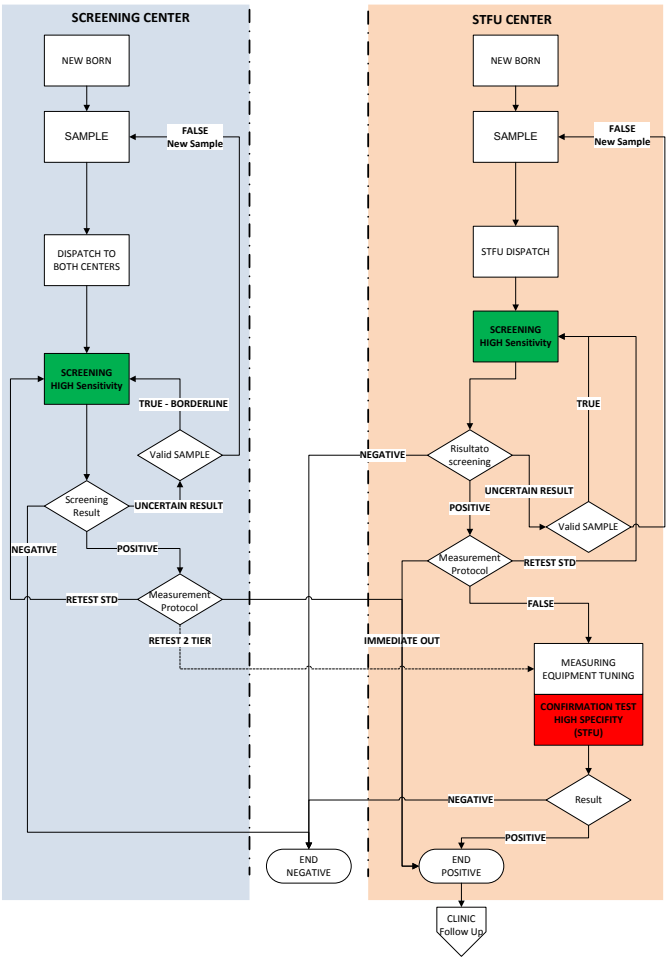

Figure 3. One structure performs high sensitivity screening, the other performs high specificity retest too.

The second solution described in Figure 3 represents the configuration where one of the two centres available in the considered geographical area is able to perform both high sensitivity and high specificity test. This is the typical case where to a centre with structures and personnel able to perform 2-tier screening a new centre able to perform only high sensitivity screening for its own area is associated.
This configuration is frequently identified in the literature by remarking that one of the centres either “performs the Short Term Follow Up function”, or “has the ability of performing high specificity analysis”, and consequently has a better predictive ability.
Note that if the second tier centre has only one machine at disposition for analyses, such a machine may be utilized for both high sensitivity and high specificity analysis, therefore it is necessary to consider the new tuning activity to get a different machine setting; such an activity may be effected either when necessary or periodically (for instance once a week) with different results for what concerns the overall sample crossing time (both for negative and positive ones). Another factor affecting the mean sample crossing time is the necessity of transferring the sample to the centre able to perform high specificity analysis whenever the high

sensitivity analysis has given positive result in the other centre: to face the related difficulties it is generally suggested to send the samples simultaneously to both centres, when they are attributable to the centre performing only high sensitivity analysis.
A final notation about the model concerns the adoption of an "immediate output", with exit towards the clinical diagnosis and possible therapy even in absence of MS/MS retest, in case the sample results to be positive with respect to some pathologies associated with very rapid clinical evolution.
Advantages presented by this model are the following:

- The amount of false positives is reduced;
- It is possible to manage the cluster in a centralized and coordinated way (for instance for trigger setting);
- It is possible to specialize teams and organization in a different way in the two centres.

The only disadvantage is the increasing of sample crossing time.
This method is valid if in the catchment area where the two centres are competent a team with greater experience in the management of metabolic diseases is present, to which the whole cluster is
entrusted.

3.4 Cluster with geographical distribution of population on the specific competent centre, where both structures perform both high sensitivity screening and high specificity retest when applicable.
The third solution described in Figure 4 represents the configuration where both centres independently effect the whole test-retest path with different setting on the catchment area where they are competent. Actually it represents a system of independent centres utilizing a method which permits to reduce the amount of false positives addressed to the successive phase of clinical diagnosis. The hypothesis of considering the two centres pertaining to an only cluster is represented by the fact that all decision elements (for instance, trigger values) are ruled by an only direction.
The advantage with respect to the previous model is given by a substantial reduction of sample crossing times.
The disadvantages are the following:
- In order to operate correctly the structures shall work for a greater amount of time;
- In both structure special personnel, able to effect machine tuning and with greater experience in reading screening results, is necessary.

3.5 Centres with differentiated purposes
The difference of this model, described in Figure 5, with respect to the previous ones stays in the fact that centres perform activities which are only partially superimposable, as they are devoted to develop different activities and specializations, related to a high sensitivity and a high specificity structure.
Samples are allocated to centres not on the basis of geographical origin but according to different criteria; in the

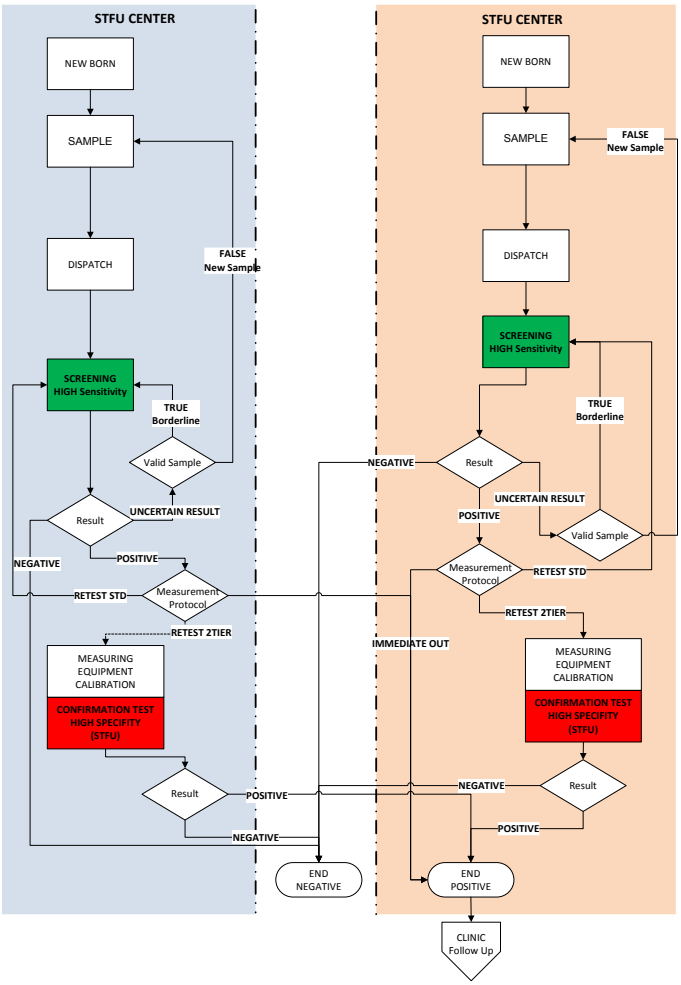

Figure 4. Both structures perform high sensitivity screening and high specificity retest.

model hypothesis all samples are simultaneously sent to the two centres (double sample and double sending) except for those which belong to the "special protocol" category (second samples in case of uncertain result, preterm and underweight infants, familial or ethnic recurrences, etc.) which are directly sent to the high specificity centre.
The principal benefit of this approach lays in permitting to both centres structures to build up a sufficient experience to reach a high qualitative standard. In fact:
- the high sensitivity centre gets a very large amount of samples, corresponding to a larger catchment area with respect to previous models; anyway that is manageable because this centre has to operate only high sensitivity screening and to communicate to the high specificity centre the reference of samples on which the retest is to be effected (possibly with different machine tuning); the high sensitivity centre operation is effected without off-line or non serialized activities;
- the high specificity centre, devoted to the analysis of samples requiring retest and to protocols requiring non standard treatments, on the contrary has a much smaller amount of samples to analyze; therefore it can examine a

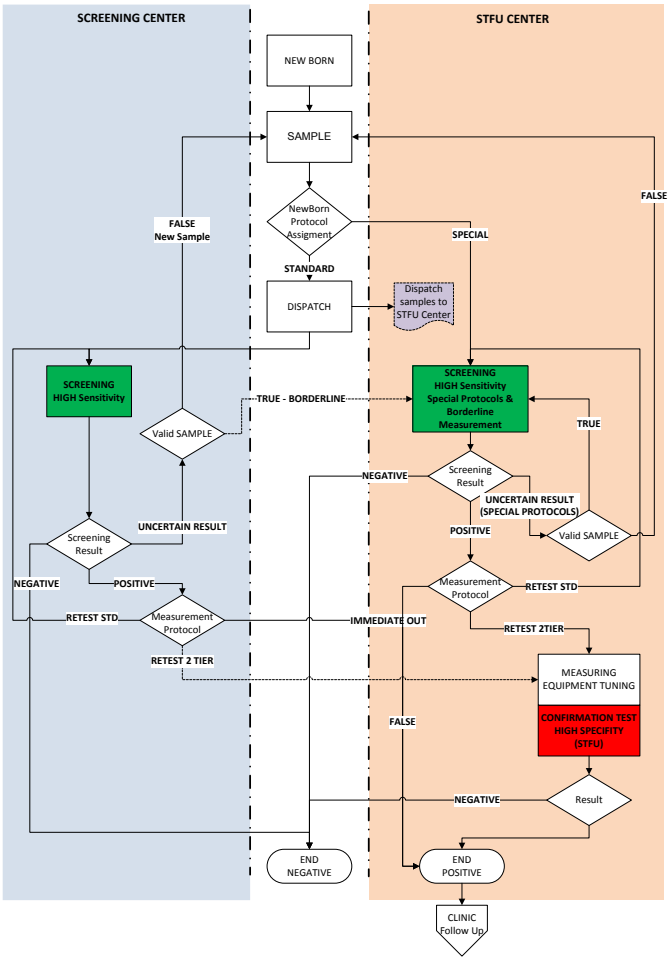

Figure 5. Centres with differentiated purposes.

large amount of important samples following ad hoc procedures without an excessive increase of crossing time, as they are managed by a machine able to work much smaller lots, sometimes of only one sample.

Therefore this model, differently from other suggested configurations, permits to utilize different settings for the machines of the various structures, also in a permanent way and without requiring any tuning. Then the screening centre may be set with a configuration devoted to false negatives reduction (high sensitivity centre); the false positives amount will be obviously larger with respect to what is commonly given in the literature; the hypothesized structure is sufficiently dimensioned also with a comparatively large birth catchment area (100,000 units per year). On the contrary the second structure of the cluster system may be adapted to a greater specialization, as it has to manage a smaller sample amount, and such samples are always important, as either they come from newborns belonging to particular groups or they have been indicated as positive by a first screening.

This double structure (screening and confirmation) with contextual availability of blood samples has the advantage of effecting off-line all tests necessary to reduce the chance of false positives without stopping the routine operation of the other centre (with consequent machine tuning); such a way useless addresses to clinical diagnosis are avoided, with consequent benefit to the parents' mental health.

The main advantages are the following:

- This configuration permits an optimal sample distribution in terms of analysis quality (building of personnel exper anience, territorial personnel distribution so to avoid duplicates, specialization of personnel devoted to positivity confirmation;
- This model is easily scalable as it permits to dimension the catchment area with respect to one or more high sensitivity screening centres with a reference centre for high specificity analyses;
- With respect to previous models a substantial reduction of positive sample crossing times is reached, above all in the hypothesis of having permanently machines with differentiated tuning.

The main disadvantage is due to costs of double withdrawal and double sending.

4 SIMULATION MODEL

In order to reach the scopes indicated in the introduction we built up a simulation model, coded in language Rockwell Arena, able to describe simultaneously the four presented conceptual models.

4.1 Macroscopic description

Here we present in Figure 6 a macroscopic description of the simulation model by means of submodels; from left to right we see:

1. Sample input in the system (coinciding with births);
2. Determination of positive samples (successively detected) and of special protocols;
3. Sample dispatch to the competent centre (after sample doubling in order to let the four different models operate simultaneously);
4. Models corresponding to the four conceptual solutions seen in Section 3.

Sample inputs are scheduled according to a Poisson process.

Consider now what represented in Figure 7. In the considered conceptual models a sample, after withdrawal and transport, proceeds to the test and get either positive or negative result, as shown to the left in the figure. In simulation, in order to increase clarity and data manageability, the procedure shown to the right in the figure is adopted; to every sample either a pathology from the panel of considered ones or no pathology is probabilistically allocated, i.e., either a positive attribute to one disease or no attribute is assigned, and consequently the probability of all possible results is given, both related to high sensitivity screening only and related to high sensitivity screening plus high specificity confirmation; such a way the test result is deterministically obtained later, by knowing whether the single or the double test has been applied.

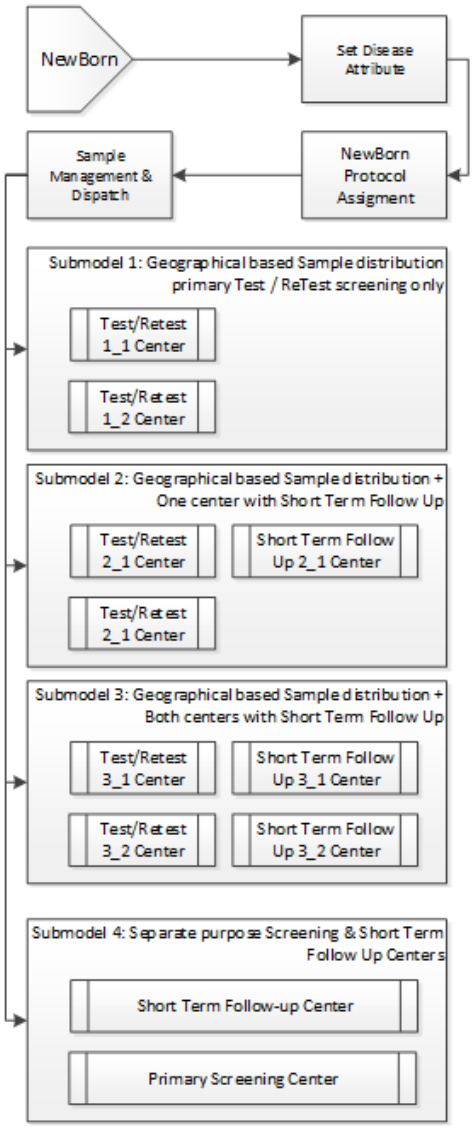

Figure 6. Simulation model macroscopic description

By a probabilistic multiple decision all non standard protocols (preterm, underweight, special) are defined by a further attribute.
All above probabilities are obtained either from the literature or from direct experience on the actual situation.
Sample transportation by an actual carrier with related time constraints is simulated.
Finally the four alternative models, describing the above mentioned alternative configurations, are coded in Arena, reproducing the corresponding conceptual models with actual operation parameters. The whole Arena model is reported in Figure 8.

4.2 Simulation results

The simulation results, related to an actual situation and to every cluster type, include:

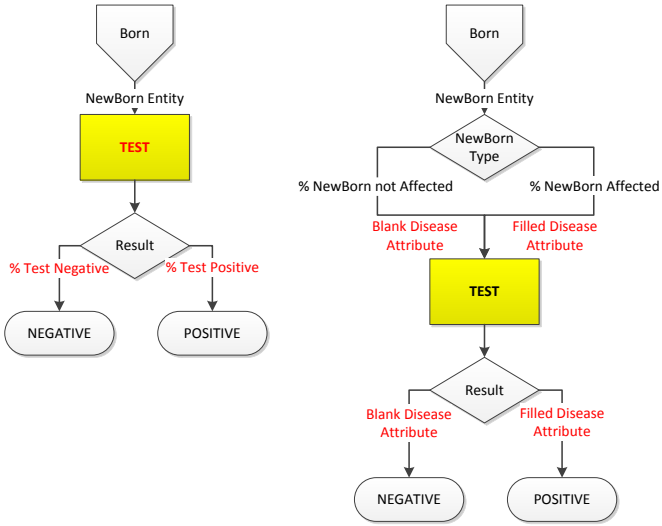

Figure 7. Conceptual and simulation oriented sample parametrization.

- Crossing time: for every pathology it is checked whether the crossing time is acceptable with respect to the risk of delays in the diagnosis and therapy phase with consequent permanent damages to positive newborns;
- Workload: both workload and performance of test structures (machines, personnel, etc.) and workload of clinical structures devoted to diagnosis and therapy are computed.

The above considered results may be usefully studied to compare possible solutions and to suggest a correct dimensioning for the chosen configuration.

4.2 Simulation model applications

We have to remark that data from the literature about metabolic hereditary diseases incidence and related detectability, based on screenings performed in various countries, are notably different from one another. This is due to two different causes: the first is connected to population characteristics, the second to cut-off values adopted to distinguish positive and negative patients from tandem mass spectrometry results. Therefore the screening process can be optimized only after a sufficiently large time interval (generally at least two years), to know incidence parameters with good precision. In the meantime we may only base our actions on a value interval, between minimum and maximum values taken from the literature.
The model has been used to rightly dimension the screening process in Veneto Region (North-East Italy), where two screening centres are set. In correspondence with minimum and maximum incidence values from the literature, and for different cut-off values, the amount of positive patients both non detected and non treated in time has been evidenced by running the proposed model; as a consequence the operating rules for both screening centres (working time for every day of the week) and clinical follow-up centres (number of patient individual visits and investigations) have been

obtained to minimize the probability of damaged patients. As a first application, the first configuration (cluster with geographical population distribution, only high sensitivity screening), was considered. As useful alternatives, all other configurations, with related results corresponding to different adopted cut-off values, were presented and will be taken into consideration by regional health care managing authorities. In the meantime, local results will permit to refine population based characteristics in term of diseases incidence.

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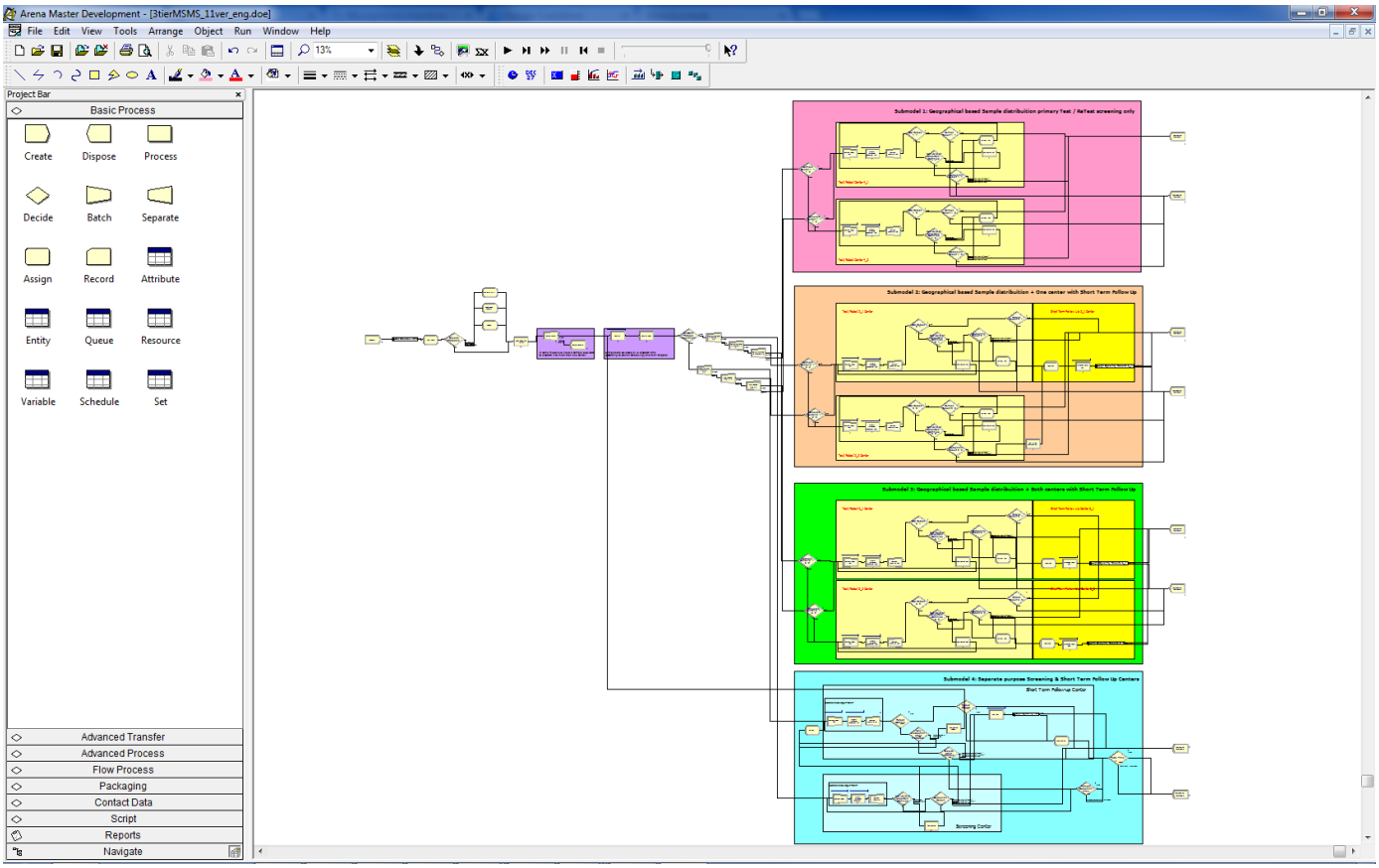

Figure 8. Complete Arena model