pipette
SWISS LABORATORY MEDICINE

Pipette 1-2019, Seite 21-22

White paper on molecular diagnostic panels in microbiology

Molecular diagnostic panels (MDx panels, thereafter panels) allow to test for several pathogens such as viruses, bacteria and parasites within a single syndromic-driven diagnostic approach. Clinicians increasingly use these tests due to their ability to rapidly evaluate patients presenting with unspecific symptoms with e.g. diarrhea or respiratory tract infections. Exclusion or inclusion of specific pathogens holds promises to tailor antibiotic treatments. Clinical microbiologists are therefore confronted with an increasing diagnostic demand from clinicians regarding usage of such panels. Those panels are often easy to use and reliable, based on their cartridge design, with the downside that their costs are high and that the knowledge on the diagnostic targets of these assays are often undisclosed.

The Commission of Clinical Microbiology of the Swiss Society for Microbiology decided to analyze this topic in further details. Despite tremendous diagnostic potential, the indiscriminate use of these panels could be challenging at several stages of the diagnostic process. Pre-analytical challenges include the high costs of many of those assays. Panel assays should therefore be carefully used in well-defined groups of patients e.g. severely ill or immunosuppressed. A key aspect of the diagnostic stewardship consists of providing recommendations towards proper assay usage in clinics. At the analytical stage, the parallel format of these panels implies specific quality control strategies. In the post-analytical part, the availability of results that are not prescribed nor even foreseen by the clinician raise additional interpretation issues. Profound knowledge on the employed panels helps to interpret unexpected positive results as well as to address the poor sensitivity for detecting specific pathogens. Finally, the clinical impact of many panels has not been evaluated in great details, to properly support their costs.

The Commission of Clinical Microbiology discussed these aspects with the objective to provide a white paper to the Swiss clinical microbiologists, trying to capture the essence of this technique. The objective is to help framing panel implementation, mostly by guiding QC and results interpretation. We also underline the urgent need for clinical studies using these panels, with a literature that remains clearly incomplete and heterogeneous, in order to assess the true performance and clinical impact of each of these multi-target assays.

It rapidly appeared impossible to provide sufficient and adequate literature review, nor have enough Swiss data on the different available systems, due to the rapid development of panels and the incomplete literature, especially regarding local epidemiology.

Therefore, this paper will discuss the principles of competence for performing the assays, their clinical utility and clinical validation, their QC and results interpretation.

1. **Competence**: These molecular assays have -by law (Epidemics Act, SR 818.101, art 16)- to be performed in a laboratory, typically in an authorized microbiology laboratory. However, stat labs or core labs could be considered as adequate laboratories for performing these assays provided there are:
   i) urgent assays (e.g. Group B Streptococcus detection),
   ii) a documented training and a QC program, and
   iii) that these analyses are always under the responsibility of a microbiologist. A specific attention should be paid to the interpretation of the results, as explained below.

2. **Qualification**: Any new panel on the market should be first qualified by a laboratory/laboratories to assess its quality in terms of sensitivity and specificity. An independent publication should be available. A national validation study should be considered based on the competence of the lab(s), recognition from the community and from the manufacturer. A final report would enable laboratories to appreciate the quality of the test and to better define its intended use.

3. **Clinical utility**: Microbiologists should advise clinicians on the best situation for using each panel in order to increase their diagnostic yield. As an example, a gastroenteritis panel could be recommended for patients suffering diarrhea when coming back from tropical regions; or as a second line assay in case symptoms persist despite frequent causes have been already ruled out. In contrast, there is often no rationale to perform a respiratory panel in a non-immunocompromised otherwise healthy patient. Ideally, physicians requesting the use of panels should give adequate justification (e.g. travel abroad in regions with poor hygiene for GE panels or compromised immunity for respiratory panels). Furthermore, panels should not be used on clearly inadequate specimens (e.g. non-diarrheic stools or sputum specimens contaminated with saliva). The simultaneous presence of

several pathogens in a specimen submitted should prompt a phone call to the requesting physician to discuss the results. Finally, the lower sensitivity of some assays has to be considered. Bordetella pertussis is a perfect example, as adults often visit their physicians later during the disease (e.g. after ten days of symptoms). In this case, an insufficient sensitivity of the panel can lead to a false negative result, whereas a classical PCR would correctly address the question. The clinician needs therefore to be able to interpret the test results to make a proper medical decision and truly benefit from the speed of the panel, justifying its costs.

4. **Microbiological validation**: Introducing a CE-marked assay might not require an extensive validation of the panel, but a verification should nevertheless be done in order to test the own laboratory work-flow, the interface with the laboratory information system and to have a positive control available in case of a problem with the test system. Many of the newest panels have assays to detect very rare pathogens and not all laboratories can completely evaluate all targets of a panel e.g. tropical pathogens, or viral pathogens such as MERS coronavirus. Reference laboratories should be contacted in order to assess and properly interpret such samples. At least initially, a representative number of specimens negative by non-panel assays or routine methods should be run with the panel to detect major problems of specificity. Positive results should be confirmed by an independent method (e.g. culture) for an adequate number of specimens in daily routine. Excessive numbers of non-confirmed results should prompt further investigations. Although the assay manufacturer provides recommendation on which transport media should be used, laboratories are often confronted with real-life issues such as the usage of not validated transport media or sample types. Laboratories may decide to test the most commonly received transport media and sample types in order to become familiar with the assay performance. In case of use of a non-validated transport medium, a commentary should be added to the results.

5. **Clinical validation**: Only very few assays have been evaluated in large multi-center studies and have documented a clinical impact on patient management. Clinical validation should include: (i) the laboratory performance with e.g. turn around times, (ii) sensitivity and specificity comparison with the laboratory internal gold standard, and (iii) the impact of the fast test result on treatment change or

even clinical outcomes. This yields a series of challenges –the comparison against the laboratory gold standard is often not easy – as the broad range of pathogens covered by many panels does often not allow for a single confirmation of all pathogens. Also the prospective determination of a patient outcome requires an informed consent from the patient, even if a panel assay can be considered a more advanced diagnostic approach than e.g. culture-based diagnostics. Thereby new panels can hardly be evaluated as the patient consent does significantly delay complete panel evaluation: tests are then often simply introduced without proper evaluation. Nevertheless, diagnostic laboratories should try to evaluate the impact on the clinical usage.

6. **EQC**: to compare labs, by using test probes that are provided independently from the manufacturers. To be ideally run 2x/year on 8-12 samples but based on what is commercially available. Each separate EQC will not test all targets of the MDx panel (like for cultures!), but one expects the EQC to ultimately assess each target, after several EQC distributions. A problem is that EQCs often are not specified for a single test – therefore not all samples are suitable for the quality control. In addition, the high assay costs make it very expensive to test multiple EQCs to cover all pathogens within the test.

7. **IQC**: to assess assay robustness under routine conditions. IQC should be rotated on the various slots of the device to ensure similar performances (like automated blood cultures!). To be performed for each new lot (or for each delivery, if transportation could affect robustness). Assess cartridge stability using the last cartridge of the lot, in case of bad storage only.

8. **Data interpretation**: as usual, predictive positive and negative values are tightly related to the prevalence of the targeted disease. This aspect is of peculiar importance here, due to the parallel nature of the panel that can deliver unexpected positive results. The latter should therefore be interpreted in the light of the prevalence of the disease, to translate these results into meaningful positive predicted value. Let's consider few examples hereafter:

    a. An elderly person suffering diarrhoea without travel history is highly unlikely to suffer from cholera. Therefore, a molecular

result suggesting the presence of Vibrio cholerae is to be considered as a false positive and returned as such to the physician (by providing a comment and a call to the prescriber, most likely). However, a similar finding in a traveller should prompt a clinical suspicion, and has to be investigated by a more detailed patient history and additional microbiological tests if the suspicion persists. Some results should always trigger confirmatory tests, call to the prescriber and warrant additional clinical evaluation (e.g. Vibrio cholerae, MERS-Cov, HHV-6). Quantification, results using a second method, referring the sample to a reference center or even to the manufacturer should or could be requested.

b. The observation that positive EHEC results from stool panels often do not have clinical relevance (true positive biological result – false positive clinical result) due to carriage detection in a patient without suspicion of EHEC. This phenomenon has become obvious with the broader use of MDx panels since this specific EHEC assay would not have been performed otherwise, in the absence of clinical suspicion. Laboratories should therefore develop strategies to mitigate such situation, either by masking those results that were not directly requested by the physician, or by calling the physician to discuss their interpretation.

9. **Post-market surveillance**: Users should be encouraged to rapidly notify any problem encountered with such panels, so that unexpected events (false positive, false negative) could be accessible to all users, through an open communication to and from the manufacturers. One might therefore expect manufacturers to directly disclose such events or maintain a publically accessible and daily updated database to their users. Users have to report incidents (defined by law) to Swissmedic either directly or via a Materiovigilance Contact Person or a professional association (https://www.swissmedic.ch/swissmedic/en/home/medical-devices/reporting-incidents---fscas/users---operators.html).

10. The following elements should be carefully considered when using MDx panels:

a. The prevalence of a disease may vary markedly across time and geography (e.g. cholera in Switzerland versus India). The same panel contains probes for diseases of markedly different frequencies (e.g. rare cholera and frequent Campylobacter in Switzerland, especially during holiday season and consumption of Chinese fondue), leading to very heterogenous performance due to highly different a priori probabilities.
b. The limitations mentioned above are typically considered by a physician when ordering an assay. The use of a panel biases this risk balance, as evidenced by the –way too frequent- reporting of EHEC, that are simply not clinically relevant and that were not thought of or even requested by the physician.
c. Finally, each assay on a MDx panel has its biological performances that should be known by the microbiologists.
d. Interpreting a result from a molecular panel represents therefore a tricky task, that has to integrate the clinical a priori probability and the assay performance, for returning meaningful and medically actionable decisions.

Authors (alphabetic order): André Burnens, Adrian Egli, Hans Fankhauser, Meri Gorgievski, Nadia Liassine, Gladys Martinetti, Martin Risch, Hans Siegrist, Marie-Lise Tritten, Reinhard Zbinden and Jacques Schrenzel.

References

1. Humphries RM, AJ Linscott. 2015. Laboratory Diagnosis of Bacterial Gastroenteritis, Clin. Microbiol. Rev. 28: 3-31
2. Shane AL, RK Mody, JA Crump, PI Tarr, TS Steiner, K Kotloff, JM Langley, C Wanke, C Alcantara Warren, AC Cheng, J Cantey, LK Pickering .2017. Infectious Diseases Society of America Clinical Practice Guidelines for the Diagnosis and Management of Infectious Diarrhea. Clin. Inf. Dis. 65:e45–e80
3. Smieja M, DM Goldfarb 2016. Molecular Detection of Diarrheal Pathogens. Clin. Microbiol. Newsl. 38: 137-145

4. Binnicker MJ. 2015. Multiplex Molecular Panels for Diagnosis of Gastrointestinal Infection: Performance, Result Interpretation, and Cost-Effectiveness. J. Clin. Microbiol. 53: 3723-3728
5. Ramanan P, AL Bryson, MJ Binnicker, BS Pritt, R. Patel. 2017. Syndromic panel-based testing in clinical microbiology. Clin. Microbiol. Rev. 15: 1-28.