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Performance and Cost Efficiency of KRAS Mutation Testing for Metastatic Colorectal Cancer in Routine Diagnosis: The MOKAECM Study, a Nationwide Experience

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Abstract

Purpose: Rapid advances in the understanding of cancer biology have transformed drug development thus leading to the approval of targeted therapies and to the development of molecular tests to select patients that will respond to treatments. KRAS status has emerged as a negative predictor of clinical benefit from anti-EGFR antibodies in colorectal cancer, and anti-EGFR antibodies use was limited to KRAS wild type tumors. In order to ensure wide access to tumor molecular profiling, the French National Cancer Institute (INCa) has set up a national network of 28 regional molecular genetics centers. Concurrently, a nationwide external quality assessment for KRAS testing (MOKAECM) was granted to analyze reproducibility and costs.

Methods: 96 cell-line DNAs and 24 DNA samples from paraffin embedded tumor tissues were sent to 40 French laboratories. A total of 5448 KRAS results were collected and analyzed and a micro-costing study was performed on sites for 5 common methods by an independent team of health economists.

Results: This work provided a baseline picture of the accuracy and reliability of KRAS analysis in routine testing conditions at a nationwide level. Inter-laboratory Kappa values were >0.8 for KRAS results despite differences detection methods and the use of in-house technologies. Specificity was excellent with only one false positive in 1128 FFPE data, and sensitivity was higher for targeted techniques as compared to Sanger sequencing based methods that were dependent upon local use of in-house technologies. Specificity was excellent with only one false positive in 1128 FFPE data, and sensitivity was higher for targeted techniques as compared to Sanger sequencing based methods that were dependent upon local use of in-house technologies. Estimated reagent costs per patient ranged from €55.5 to €190.9.

Conclusion: The INCa has set-up a network of public laboratories dedicated to molecular oncology tests. Our results showed almost perfect agreements in KRAS testing at a nationwide level despite different testing methods ensuring a cost-effective equal access to personalized colorectal cancer treatment.
Introduction

New therapeutic approaches such as anti-EGFR targeted therapies and concurrent identification of molecular biomarkers to identify sub-groups of potentially responsive tumors had created a need for routine molecular characterization of cancers. In colorectal cancer, the demonstration that patients with KRAS mutated tumors did not benefit from anti-EGFR monoclonal antibodies was established independently of the technology used to identify KRAS mutated tumors [1]. This result was rapidly followed by a directive of the European Medicines Agency (EMEA) that restricted the use of cetuximab (Erbitux®) and panitumumab (Vectbi®) to patients with KRAS wild-type metastatic colorectal cancer [2].

With more than 940,000 new colorectal cancer cases worldwide each year, the use of anti-EGFR targeted therapies are faced with main issues, an economical one: who pays for the test or the drugs and a medical one: who performs the test? The French public health insurance system decided to provide targeted therapy for colorectal cancer in line with the EMEA recommendation. In parallel, the French government and the National Cancer Institute (INCa) have set up a national network of 28 regional molecular genetics centers to implement routine molecular testing for colorectal cancer. More than one laboratory can be related to one regional center.

Each laboratory developed KRAS testing according to its own expertise and to the locally available instruments. The number of tests increased from 1,100 in 2007 to 10,012 in 2008 and 17,246 in 2009. From then on, the number of tests was stable and covered the expected incidence of metastatic colorectal cancer patients in France. A founding of €2.5M was devoted to KRAS testing. This organization seemed cost-effective considering global gain on drug costs. It was necessary to prove that KRAS testing results were reproducible between molecular laboratories. Each laboratory using one or more genotyping method was evaluated by an external quality control program, the multicenter program: KRAS Oncogene Mutation detection in the treatment of Metastatic Colorectal cancer by EGFR Antibodies (MOKAECM). The MOKAECM project was set up as an external quality control and laboratories were free to choose and develop their own method for KRAS testing.

Previous comparative studies evaluated one technology [3] [4,5]. Others compared different techniques with one tested technology per site. In both cases the robustness of a technology used with different levels of expertise cannot be evaluated [6] [7]. A national assessment of KRAS mutation testing linking actual practices associated with cost evaluation has never been done up to now.

The first objective of the MOKAECM project was to evaluate at a nationwide level the performance of KRAS testing for clinical purpose (sensitivity and reproducibility). The second was to estimate and compare the costs associated to each technology. As this study covers a national territory including all the INCa labeled molecular laboratories, we may infer the national performance for KRAS testing from the MOKAECM study.

Materials and Methods

Study Design

This study was designed to evaluate KRAS genotyping in 40 French laboratories related to one of the 28 molecular genetics centers, using cell line and formalin-fixed paraffin-embedded (FFPE) tumor samples. ADNs were centrally prepared to control homogeneity and blindly sent to all participants for KRAS testing using routine practice technologies. Results were loaded and stored in a specific database and analyzed by a statistician (GC) from the HEGP hospital Clinical Research Unit.

Cell Lines

ATCC Cells lines (H1573:p.G12A; H358:p.G12C; A427:p.G12D; LS123:p.G12S; SW620:p.G12V; Lovop,G13D; SW46:Wild Type) were specially purchased for the study and KRAS G12R, was obtained by retroviral infection of 292FT cells with a vector containing the KRAS c.54G>C substitution (JCP).

Colorectal Cancer Tissues Samples

Twenty-four tumors were characterized and selected from patients undergoing surgical resection for colorectal cancer at the Ambroise Paré Hospital, (Boulogne-Billancourt, France). The Ethics committee of Ile de France II approved the study and patients were informed and written consent was obtained according to French law. The study was conducted in France. Diagnosis of colorectal adenocarcinoma was assessed by a pathologist (JFE) who selected the FFPE blocks for subsequent molecular analysis. DNA extraction was done at the Ambroise Paré Hospital using the QIamp DNA Mini Kit (Qiagen). The tumors were characterized for KRAS by three different labs using three different technologies. These laboratories were selected based on their experience with KRAS testing and in house validation of the method used. Selected samples showed no discrepancy.

Assessment of Cellularity

The evaluation of cellularity was performed at Hematoxylin, eosin and safran (HES) slides at both ends of the FFPE block used for DNA extraction. Slides were scanned on a Mirax Scan, (Zeiss, Göttingen, Germany). To validate the tumor cell content, the scanned images were reviewed by seven independent pathologists from different centers. When discrepancies were noticed, slides were reviewed and consensus was found.

Methodology Used

Different in-house methods were developed: Sanger direct sequencing (n=15 laboratories), allelic discrimination probe
systems [8,9] (n = 13), Snap Shot [10] (n = 7), pyrosequencing [11] (n = 5), HRM followed by sequencing [12] (n = 5). One laboratory used the TheraScreen®- K-RAS Mutation Kit commercial kit. Five laboratories tested more than one method.

Thirty laboratories brought their entire protocol for KRAS mutation detection, there was no practice homogeneity except for laboratories using KRAS TaqMan® probes (see Information S1). Detailed procedures with primers positions are available on request.

**Statistical Approach and Data Analysis**

Error rate was defined as the sum of false positives, false negatives, non-contributive tests and wrong mutation calling. To fit to the criteria used by the European EQA, all errors were at first considered equally significant although the implication for patients may differ. All samples were selected to have no amplification default and each participant submitted a result that we considered as being the final report sent to the oncologist. In a second step we considered clinically relevant errors as being false positive and negative results considering that in case of failure a new sample would be requested although this will result in a further delay for the patient.

Success rate was defined as the sum of true positives and true negatives.

We assessed both reproducibility (inter- and intra-laboratory) and diagnosis accuracy (sensitivity and specificity) of the different techniques for KRAS genotyping. For each mutant cell line, there were four different dilutions (5%, 20%, 50%, 100%) with three replicates per dilution. All the data were taken into account.

For the six techniques, used by at least two laboratories, inter-laboratory reproducibility was assessed using a generalization of the Cohen Kappa statistics for measurement of agreement among laboratories – with m ranging from 2 to 15 according to the technique- into one of the eight mutually exclusive categories. As there were failures (inability for the technique to give a mutation status), the calculation taking into account missing data. Confidence intervals for the true generalized Kappa coefficient were computed using the bootstrap resampling method, to take the intra-cluster correlation into account [14].

For assessing intra-laboratory reproducibility for a given KRAS genotyping technique, we computed a Kappa statistic for each laboratory, as described above, based on the triplicate aliquots. Then, we summarized the results by providing average Kappa coefficients, with the range of Kappa coefficients across laboratories.

Diagnosis accuracy for the detection of each specific mutation (categorical gold standard) was assessed for each technique and each laboratory. Materials used in the two rounds can be considered as gold standard materials (cell lines, validated tumor DNAs). It was possible to assess a sensitivity and specificity for each laboratory with cell line material and FFPE DNA samples. For each technique, sensitivity and specificity for the detection of mutation (binary gold standard) were computed by combining data from all laboratories. A ratio estimator for the variance of clustered binary data which takes intra-cluster correlations into account was used for calculating 95%CI [15]. All analyses were performed using the SAS software version 9.1.

**Economical Assessment**

Five technologies were compared: “Direct sequencing”, “SNaPshot”, “Pyrosequencing”, “High Resolution Melting” (HRM), “TaqMan®”. Costs were estimated from the point of view of the laboratory by microcosting and time-motion studies. We estimated fixed and variable costs associated with each of the five technologies: labor, consumables (i.e. reagent and others consumables) and equipment and excluded overheads. Purchase price was used for supplies and equipment with a 5-year linear amortization and labor was valued using total payroll [16].

**Cost Sensitivity Analysis**

A sensitivity analysis was led to get a range of cost by moving different parameters. The parameters were on prices (5% and ±10%) and laboratory number of acts (Information S1).

**Results**

**Analysis of Cell Line Results**

Ninety-six cell-line DNA samples were sent to 40 French laboratories, five laboratories used two different screening methods leading to 4320 reported results. Since 6 laboratories could not technically detect the p.G12R mutation (Information S1), the p.G12R samples were not taken into account and 3780 results were finally analyzed. Results were compared to the expected

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Table 1. Diagnostic value comparison between methods cell line DNA analysis.

|                | Labs (n) | Samples (n) | Analytical failures (n) | Success rate in true negative (%) | Success rate in true positive (%) |
|----------------|---------|-------------|-------------------------|-----------------------------------|----------------------------------|
| Dilutions      |         |             |                         |                                   |                                  |
|                |         |             |                         | All 100% 50% 25% 5%               |                                  |
| Direct sequencing | 15      | 1260        | 4 (3%)                 | 98.9                              | 76 99 99 87.0 38                  |
| Taqman         | 8       | 672         | 11 (1.6%)              | 99.0                              | 92.3 95.8 100 99.3 76.4          |
| Snapshot       | 7       | 588         | 4 (7%)                 | 98.8                              | 89.7 95.2 100 93.7 73.8          |
| Pyrosequencing | 5       | 420         | 6 (14%)                | 95                                | 96.6 100 100 100 89.7            |
| HRM and sequencing | 5      | 420         | 0                      | 100                               | 78.0 100 98.9 88.9 40.0          |
| MASA           | 1       | 84          | 0                      | 100                               | 92.5 100 100 100 72.2            |
| Scorpion       | 1       | 84          | 0                      | 100                               | 100 100 100 100 100              |
| HRM+Taqman     | 1       | 84          | 0                      | 100                               | 100 100 100 100 33.3             |
| PNA* based methods | 1+1    | 168         | 0                      | 100                               | 100 100 100 100 100              |

*PNA Peptide nucleic acid; *PNA was used with taqman probes or with allele specific PCR.

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Table 2. Intra and Inter-laboratory reproducibility on cell line DNA.

| Labs (n) | Samples (n) | Intra-laboratory Kappa score (CI 95%) | Inter-laboratory Kappa score (CI 95%) |
|----------|-------------|-------------------------------------|--------------------------------------|
| Direct sequencing | 15 | 1260 | 0.93 [0.79–1] | 0.86 [0.84–0.87] |
| Taqman | 8 | 672 | 0.97 [0.92–1] | 0.93 [0.93–0.94] |
| Snapshot | 7 | 588 | 0.97 [0.97–1] | 0.88 [0.86–0.89] |
| Pyrosequencing | 5 | 420 | 0.98 [0.94–1] | 0.95 [0.94–0.96] |
| HRM and sequencing | 5 | 420 | 0.97 [0.94–1] | 0.86 [0.85–0.87] |

For techniques performed by more than 2 laboratories, sensitivity ranged from 76% to 96% and specificity from 95% to 100% (Table 1). Concerning 5% tumor samples, the lowest sensitivity was found for sequencing and HRM with an overall detection rate of approximately 40% as compared to 89.7% found for pyrosequencing. A technical failure rate of 1.6% was observed for Taqman probes due to a non-interpretation by 3 laboratories of the triplicates corresponding to p.G12V (SW480 100%) homozygous samples. Intra-laboratory and inter-laboratory reproducibility were in almost perfect agreements (>0.8 for all methods) and did not depend upon mutation type (Table 2).

Table 3. Global error rate for FFPE samples callings.

| Labs (n) | Samples (n) | Errors |
|----------|-------------|--------|
| Direct sequencing | 13 | 312 | 12(3.85%) |
| Taqman | 9 | 216 | 0 |
| Snapshot | 6 | 144 | 1 (0.69%) |
| Pyrosequencing | 7 | 168 | 3 (1.79%) |
| HRM and sequencing | 6 | 144 | 2 (1.39%) |
| Others | 6 | 144 | 2 (1.39%) |
| Total | 47 | 1128 | 20 (1.74%) |

The global error rate was 10.6% (399/3780), mainly due to false negative results (89%, 357/399). Among those, 307 tests corresponded to a percentage of tumor cells of 5% while 50 involved samples with higher tumor cell ratios. The 42 remaining errors were analytical failure (n = 25; 6%), false positive (n = 2; 0.5%) and wrong mutation (n = 15; 4%). Sequencing generated all false positive results and demonstrated a false positive rate of 0.4% (2/540). Wrong mutation callings were noted for sequencing (0.8%, 11/1260) and snapshot (0.7%, 4/588).
TaqMan were optimal within the base case. Within the worse conditions (five samples per batch and 10% prices markup) TaqMan prices per test ranged from $15.7 to $20.1. Pyrosequencing costs per test were lower than $20.1 if at least 15 samples per batch were run.

### Table 4. Costs per item and total costs per test per technique and laboratory.

| Techniques   | Sequencing | HRM | SnaPshot | Pyro-sequencing | TaqMan |
|--------------|------------|-----|----------|-----------------|--------|
| Labor (£)    | 9.9        | 8.0 | 3.9      | 6.4             | 11.4   |
| Equipment (£) | 9.7        | 7.7 | 1.0      | 1.5             | 4.3    |
| Consumables (£) | 9.8      | 10.1 | 9.6      | 6.4             | 19.0   |
| Total costs (£) | 29.4     | 25.8 | 15.0    | 13.9            | 34.8   |

Discussion

The use of anti-EGFR monoclonal antibodies is restricted to the 60 to 70% KRAS wild-type metastatic colorectal tumors, making appropriate identification of KRAS mutations a key point for...
clinical practice [17,18]. Moreover limiting the use of EGFR inhibitors to patients with wild-type KRAS may be a potential solution for cost savings [19]. To ensure testing accessibility, the INCa has granted 28 regional molecular genetics centers up to 2.5 My€ [20]. The aim of the countrywide quality control network named MOKAECM, supported by the INCa, was to evaluate the different in-house developed methods by molecular laboratories for KRAS testing in routine conditions. Here, similar cell lines (4296) and FFPE tumor samples ADNs (1132) were sent to the different participants and 5448 KRAS genotyping results were submitted and analyzed. Concerning the cell lines test, the error rate was 10.6%, the detection cutoff ranged from 5 to 20% and 86% of false negative were related to the 5% dilution. 1152 FFPE sample results were analyzed in this study, 68% of test sets (method/laboratories) correctly identified the KRAS mutational status in all FFPE samples. This result is comparable with the score reported in the European KRAS EAQ scheme (70%) [21]. Recently, for KRAS mutation analysis in colorectal cancer, arbitrary thresholds for correct KRAS mutation identification was set at 97% [22]. Considering best results for laboratories testing more than one method, the average success rate for FFPE samples was 98.5% and from 100 to 91.6% (Information S1). These scores are in accordance with the results of a study ran in 10 laboratories in Netherlands [23]. Moreover, failure to attain an overall testing event score of at least 80% was defined unsatisfactory when testing a larger number of cases in the ‘Clinical Laboratory Improvement Act’ of 1988. Taking into account results from the two testing sets 96% of French laboratories had a satisfactory score over 80% that clearly shows the quality of the KRAS testing in France despite the large panel of methods. Moreover among in the 1152 tumors tested, only 20 errors were reported. This level of error is satisfying.

From a national perspective, on 1152 tumor tests in this study, 20 errors (0.017 CI95% [0.01–0.027]) were reported with 11 of them related to a single sample. Therefore the corrected error rate was 0.7% CI95% [0.03%–0.13%] per test and per tumor. An extrapolation suggests that in France, out of the 18,000 tumors analyzed each year, 54 to 234 tumors could be misgenotyped.

Genotyping errors can result from different issues as the type of fixative, the preservation procedure, the evaluation of the tumor-cell content and finally the performances of the method used for testing. Here, DNA extraction was centralized and tumor cell content was validated by 7 independent pathologists therefore only KRAS genotyping methods were compared. All selected samples had a first validation of their KRAS status carried out by three reference laboratories using three different methods. Many different techniques, including commercially available kits, have been developed and tested but the absence of a recognized reference method makes the evaluation of new technologies a difficult task.

Cell line testing may not reflect routine practices but was used as a validation test in optimal conditions to compare the sensitivity and specificity between the different technologies. For samples with tumor cell line content over 20%, that can be considered clinically relevant, analytical results showed almost perfect agreements. For samples under 20%, results were more heterogeneous, especially for direct sequencing. In our experience, HRM prescreening did not rescue low tumor content samples. The lower sensitivity of sequencing methods as compared to others was not a surprise [24,25,26], but our results also point out that performance might depend on method optimization and level of expertise. Indeed 7 laboratories using sequencing or HRM-sequencing had a low error score (<10%) in the cell line series including 5% cell line samples and no error in the tumor series. Sensitivity seems related to a couple - method/laboratory-experience - rather than strictly to a method, therefore validation and detection cutoff must be assessed in each laboratory. When low sensitivity methods are used for genotyping, macrodissection cutoff must be adequately chosen and clear preanalytic recommendations must be given to pathologists before DNA extraction. Regardless of the detection method, mutation type could impact on sensitivity. The rate of errors was around 3% with the p.G12V to more than 20% with p.G12S and p.G12A. Allelic quantification of the 7 cell line DNAs using pyrosequencing did not give any relevant explanation to the reduced sensitivity observed for some mutations, and thermodynamic consequences in the DNA melting behavior might, in part, explain this observation. In the FFPE series the error rate was 20/1120 (1.7%) versus 399/3780 (10.6%) in cell lines, for which errors were mainly due to 5% tumor cell false negatives. The error rate was 2.6% for cell lines in similar tumor content conditions (false negative due to 5% samples excluded) suggesting that tissue fixation was not an obstacle to KRAS testing. However, fixation could slightly impact on failure levels: 0.8% (9/1120) on FFPE samples versus 0.6% on cell line DNAs (25/3780). The use of methods based on the amplification of small amplicons could be a possibility to decrease the level of non-contributive results [27]. In this study, methods based on allelic discrimination based on small fragments amplification and real time PCR demonstrated no failure against up to 4% for direct sequencing methods. Finally, in the FFPE series, 14% of the errors were found in a single sample for which tumor cell content was 50% after HES examination but the quantification of mutated alleles by pyrosequencing suggested that mutant cells could only represent 20% of all. This might in part explain the discrepancies observed for this sample but also suggests that genetic variations are not equally detected. One FFPE false positive sample was identified by allele specific amplification with a PNA blocker. It was not validated by another laboratory using similar technology and was therefore considered a false positive. Moreover the clinical value of mutated sub-clone remains to be demonstrated [20,29,30,31,32].

**Cost Estimates Limitations**

This methodology of assessment of the cost was single handed since the same independent team assessed all the technology directly in the laboratories. Five technologies were studied by microcosting in ten laboratories representing one third of all French “platforms”, which carried out 25% of all KRAS mutations tests for metastatic colorectal patients in France in 2009. Although, the level of evidence could be suboptimal, as it was based on 2 observations per technology, allelic discrimination using TaqMan probes was about two or three times less expensive than any other technology studied. Cost variation within one technology or between technologies could be due to different procedures. Indeed methods were not strictly identical, even in laboratories using similar technology, and various degrees of optimization were reported. An example was the management of testing procedure – the number of positive and negative controls, the number of replicates and the number of added steps to the procedure such as gel migration of PCR products. These extra costs were valued. Concerning cost equipment a saturation hypothesis was set at the number of positive and negative controls, the number of observations per technology, allelic discrimination using TaqMan probes was about two or three times less expensive than any other technology studied. Cost variation within one technology or between technologies could be due to different procedures. Indeed methods were not strictly identical, even in laboratories using similar technology, and various degrees of optimization were reported. An example was the management of testing procedure – the number of positive and negative controls, the number of replicates and the number of added steps to the procedure such as gel migration of PCR products. These extra costs were valued. Concerning cost equipment a saturation hypothesis was set at
house” technologies costs were much lower than commercial kits, excluding equipment and labor costs.

Conclusion
The French population was 65,027,000 of inhabitants in 2011. Twenty-eight regional molecular genetics centers covering all the territories and coordinating 46 laboratories are now involved in the analysis in 16,000 KRAS testing for metastatic colorectal cancer each year. The whole network is nationally managed by the INCa. This quality control program was the first countrywide experience with 120 similar samples being analyzed by 40 different laboratories. Our results demonstrate that, when clinically relevant results are considered 82.5% of laboratories correctly identified the KRAS mutational status in all FFPE samples. This work also suggests that, while all methods are suitable for KRAS testing with an average cost of €3 per test excluding the preanalytical steps, differences exist in terms of sensitivity and robustness. The choice of the method is likely to depend on the equipment and technical expertise available locally. This quality program provided a baseline picture of KRAS testing in France. It showed that it is possible at a reduced cost to set a nationwide program, it identified errors in testing procedures for some laboratories underlining the importance of optimization, in-house validation and quality control processes using a large panel of mutations.

Supporting Information
Information S1 Details of material and methods. (DOC)

Table S1 Shows the detailed genotypes information for FFPE samples by laboratory (N = 40). The best KRAS results were kept for laboratory using more than one technology. (XLSX)

References
1. Dahabreh IJ, Terasawa T, Castaldi PJ, Trikalinos TA (2011) Systematic review: Anti-epidermal growth factor receptor treatment effect modification by KRAS mutations in advanced colorectal cancer. Ann Intern Med 154: 37–49.
2. Bokemeyer C, Bondarenko I, Makohon M, Hartmann JT, Aparicio J, et al. (2009) Fluorouracil, leucovorin, and oxaliplatin with and without cetuximab in the first-line treatment of metastatic colorectal cancer. J Clin Oncol 27: 603–611.
3. Ogino S, Kawakami T, Brahmandam M, Yan L, Cantor M, et al. (2005) Sensitive sequencing method for KRAS mutation detection by Pyrosequencing. J Mol Diagn 7: 413–421.
4. Paleham D, Ward RL, Ap Lin V, Hawkins NJ, Hitchins MP. (2009) Implementation of novel pyrosequencing assays to screen for common mutations of BRAF and KRAS in a cohort of sporadic colorectal cancers. Diagn Mol Pathol 18: 62–71.
5. Lang AH, Druzel H, Gellers-Rhomborg S, Stark N, Winder T, et al. (2011) Optimalized allele-specific real-time PCR assays for the detection of common mutations in KRAS and BRAF. J Mol Diagn 13: 23–28.
6. Franklin WA, Haney J, Sugita M, Bernier E, Jimeno A, et al. (2010) KRAS mutation: comparison of testing methods and tissue sampling techniques in colon cancer. J Mol Diagn 12: 545–552.
7. Whitehall V, Tran K, Umaphrath S, Grieu F, Hewitt C, et al. (2011) A multicenter blinded study to evaluate KRAS mutation testing methodology in the clinical setting. J Mol Diagn 13: 557–50.
8. Didelot A, Le Corre D, Lascan A, Cazes A, Pallier K, et al. (2010) Competitive allele specific TaqMan PCR for KRAS, BRAF and EGFR mutation detection in clinical formalin fixed paraffin embedded samples. Exp Mol Pathol 92: 275–280.
9. Lecureur A, Bachet JB, Boige V, Cayre A, Le Corre D, et al. (2008) KRAS mutations as an independent prognostic factor in patients with advanced colorectal cancer treated with cetuximab. J Clin Oncol 26: 374–380.
10. Lamy A, Blanchard F, Le Pesot F, Sesboue R, Di Fiore F, et al. (2011) Metastatic colorectal cancer KRAS genotyping in routine practice: results and pitfalls. Mod Pathol 24: 1090–1100.
11. Duforet S, Richard MJ, de Fraincourt F (2009) Pyrosequencing method to detect KRAS mutation in formalin-fixed and paraffin-embedded tumor tissues. Anal Biochem 391: 166–169.

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12. Solassol J, Ramos J, Cropps E, Saiit M, Mange A, et al. (2011) KRAS Mutation Detection in Paired Frozen and Formalin-Paraffin-Embedded (FFPE) Colorectal Cancer Tissues. Int J Mol Sci 12: 3191–3204.
13. Fleiss JL, Levin B, Cho Paik M (2003) Statistical Methods for Rates and Proportions, 3rd Edition; Sons JWA, editor: Wiley. 800 p.
14. Rutter CM (2000) Bootstrap estimation of diagnostic accuracy with patient-clustered data. Acad Radiol 7: 413–419.
15. Rao JN, Scott AJ (1992) A simple method for the analysis of clustered binary data. Biometrics 48: 577–585.
16. Drummond M, Sculpher MJ, Torrance G, O’Brien B, Stoddart G (2005) Methods for the economic evaluation of health care programmes.; Press OU, editor: Oxford University Press.
17. De Roock W, De Vriendt V, Normanno N, Ciardiello F, Tejpar S (2011) KRAS, BRAF, PIK3CA, and PTEN mutations: implications for targeted therapies in metastatic colorectal cancer. Lancet Oncol 12: 594–603.
18. Lecureur A, Blous H, Laurent-Puig P (2010) Oncogenic mutations as predictive factors in colorectal cancer. Oncogene 29: 3033–3043.
19. Manel EE, Kolser JM, Vermuelem LC (2009) Clinical and economic value of screening for Kras mutations as predictors of response to epidermal growth factor receptor inhibitors. Am J Health Syst Pharm 66: 2105–2112.
20. Anonymous (2011) Getting personal Targeted therapies work, but need help to get the right one. LASO.
21. Bellon E, Ligtenberg MJ, Tejpar S, Cox K, de Hertogh G, et al. (2011) External validation of the Amsterdam KRAS/EGFR mutation testing algorithm for colorectal cancer screening in clinical practice. Br J Cancer 105: 1498–1503.
22. Drummond M, Sculpher MJ, Torrance G, O’Brien B, Stoddart G (2005) Methods for the economic evaluation of health care programmes.; Press OU, editor: Oxford University Press.
23. vouchers.
24. Heideman DA, Larkin I, Doelman M, Smif ET, Verheul HM, et al. (2012) KRAS and BRAF Mutation Analysis in Routine Molecular Diagnostics.
25. Pinto P, Rocha P, Veiga I, Guedes J, Pinheiro M, et al. (2011) Comparison of methodologies for KRAS mutation detection in metastatic colorectal cancer. Cancer Genet 204: 439–446.

26. Tsiatis AC, Norris-Kirby A, Rich RG, Hafez MJ, Gocke CD, et al. (2010) Comparison of Sanger sequencing, pyrosequencing, and melting curve analysis for the detection of KRAS mutations: diagnostic and clinical implications. J Mol Diagn 12: 425–432.

27. Kotoula V, Charalambous E, Biesmans B, Malousi A, Vrettou E, et al. (2009) Targeted KRAS mutation assessment on patient tumor histologic material in real time diagnostics. PLoS One 4: e7746.

28. Song C, Milbury CA, Li J, Liu P, Zhao M, et al. (2011) Rapid and sensitive detection of KRAS mutation after fast-COLD-PCR enrichment and high-resolution melting analysis. Diagn Mol Pathol 20: 81–89.

29. Kristensen LS, Daugaard IL, Christensen M, Hamilton-Dutoit S, Hager H, et al. (2010) Increased sensitivity of KRAS mutation detection by high-resolution melting analysis of COLD-PCR products. Hum Mutat 31: 1366–1373.

30. Huang Q, Wang GY, Huang JF, Zhang B, Fu WJ. (2010) High sensitive mutation analysis on KRAS gene using LNA/DNA chimeras as PCR amplification blockers of wild-type alleles. Mol Cell Probes 24: 376–380.

31. Mancini I, Santucci C, Sestini R, Simi L, Pratesi N, et al. (2010) The use of COLD-PCR and high-resolution melting analysis improves the limit of detection of KRAS and BRAF mutations in colorectal cancer. J Mol Diagn 12: 705–711.

32. Oh JE, Lim HS, An CH, Jeong EG, Han JY, et al. (2010) Detection of low-level KRAS mutations using PNA-mediated asymmetric PCR clamping and melting curve analysis with unlabeled probes. J Mol Diagn 12: 418–424.