CA125&CA19-9 False Negative Results Case sharing

Mónika Petrilli Alberte

IVD Spain Clinical Application Specialist

2025/4/18

CONTENTS

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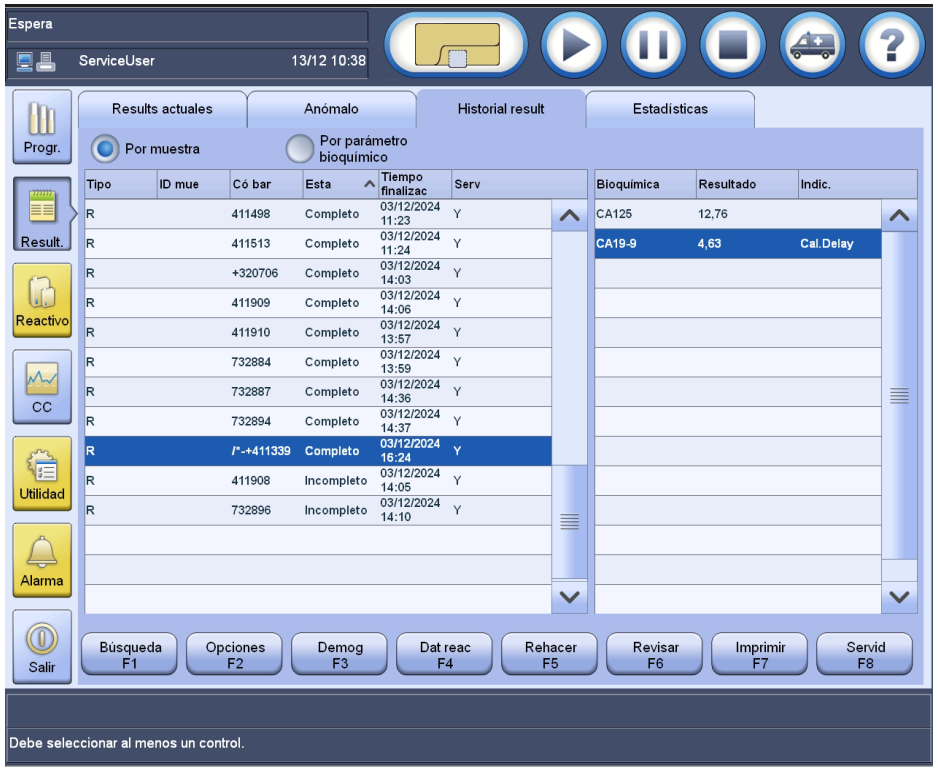
Case Background

- **Model:** Mindray **CL-1200I** .
- **Background:** an already diagnosed patient with breast cancer had to make some CA125 & CA19-9 tumor marker tests due to liver/ovarian cancer suspicion.
- **Date:** 10/12/2024

Case Background

- **Problem description:** already diagnosed cancer patient received **false negative** results for both **CA125 and CA19-9** tumor markers, even after retesting.



MARCADORS TUMORALS

DETERMINACIONS	RESULTATS	UNITATS	VALORS DE REFERENCIA
ANTIGEN CARCINOEMBRIONARI	3.64	ng/mL	No fumadors : < 5 Fumadors : < 10
Quimioluminiscència.			
ANTIGEN CA 19.9 CLIA	4.13	U/mL	[0 - 35]

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Case Ideas

Troubleshooting Ideas:

1.Check the status of analyzer, including consumables, CAL/QC results and maintenance

2. Try to remove sample interferences

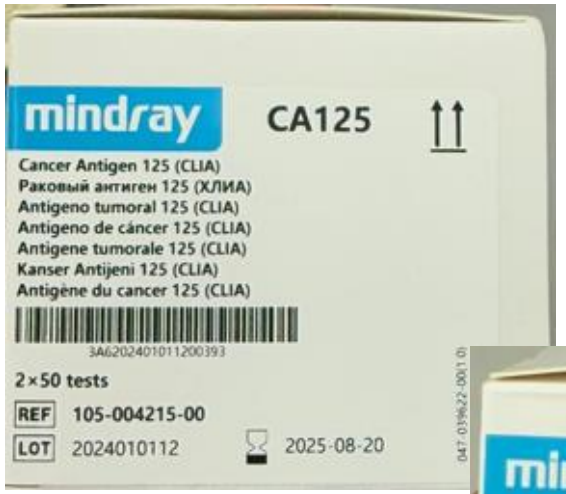
3. Method comparison

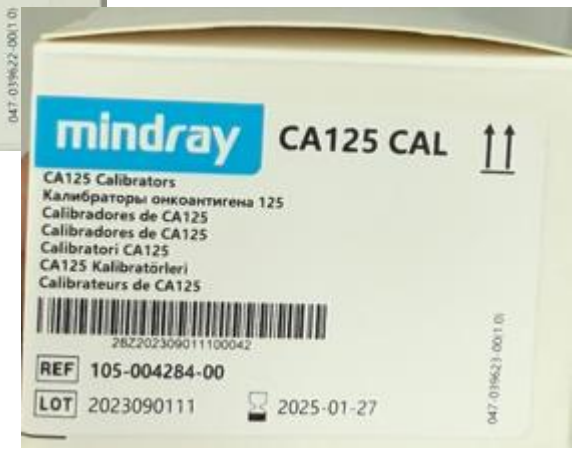
4. Clinical interpretation

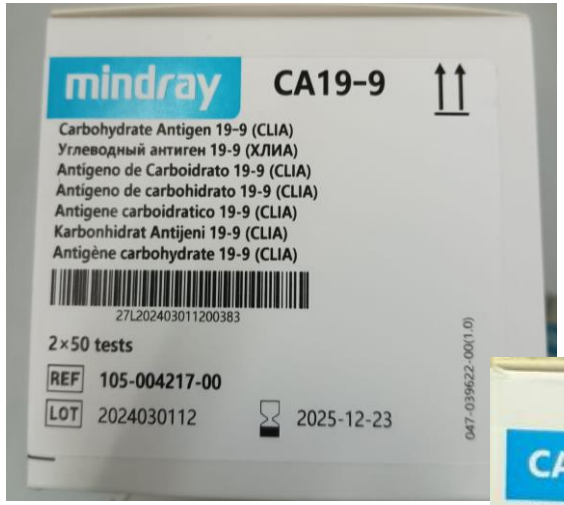
Case Ideas

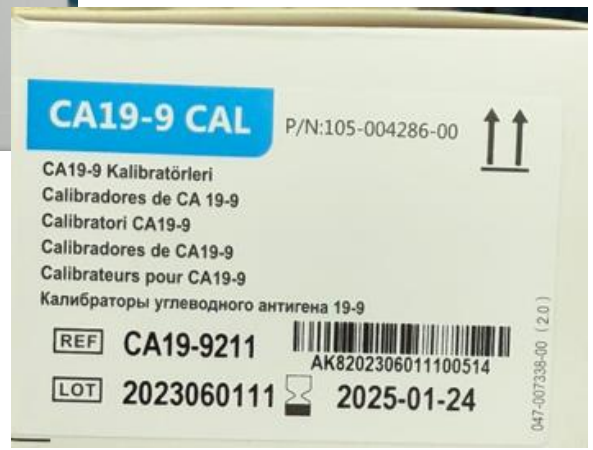
Troubleshooting process:

1.1. Check reagent, calibrator and QC materials and configuration setup









All the materials were up to date and the configuration setups were correct

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Case Ideas

Troubleshooting process:

1.2. Check calibration results

Calibration results were compared against the factory RLU values, and they were good

Datos calibrac

Químic	CA125	N.º lot r	240101	N.º ser. reac.	009413	
Modelo mat	4PLC	Estado calib	Calibrado	Fecha cal	03/12/2024 15:40:01	
Nº de lote	230901			Versión de los parámetros	V1.01.01 / V01.16	

Nivel	Concent	RLU	Indicad.	N.º lote sustrato	N.º serie sustrato	Fech/hora calib
C0	0	3411,937		20240101	00637	03/12/2024 15:38:01
C0	0	3379,986		20240101	00637	03/12/2024 15:38:21
C0	0	3407,096		20240101	00637	03/12/2024 15:38:41
C1	104,58	111886,160		20240101	00637	03/12/2024 15:39:01
C1	104,58	107910,731		20240101	00637	03/12/2024 15:39:21
C2	2048,71	2344848,725		20240101	00637	03/12/2024 15:39:41
C2	2048,71	2330408,022		20240101	00637	03/12/2024 15:40:01

Datos calibrac

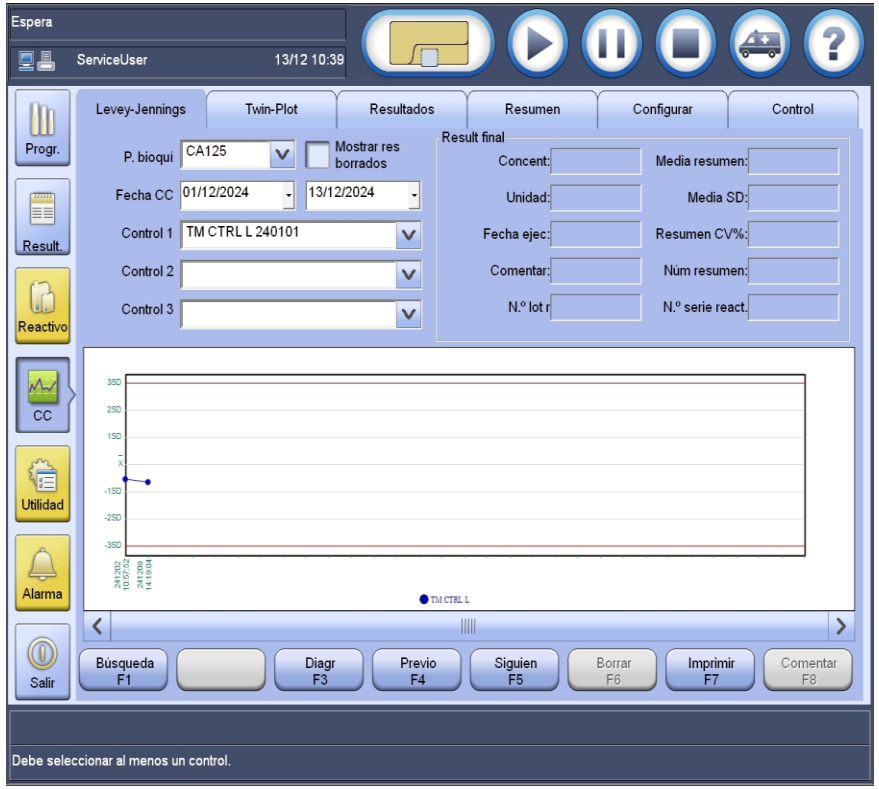
Químic	CA19-9	N.º lot r	231001	N.º ser. reac.	236197	
Modelo mat	4PLC	Estado calib	Extendido	Fecha cal	06/09/2024 14:55:35	
Nº de lote	230601			Versión de los parámetros	V1.02.01 / V01.05	

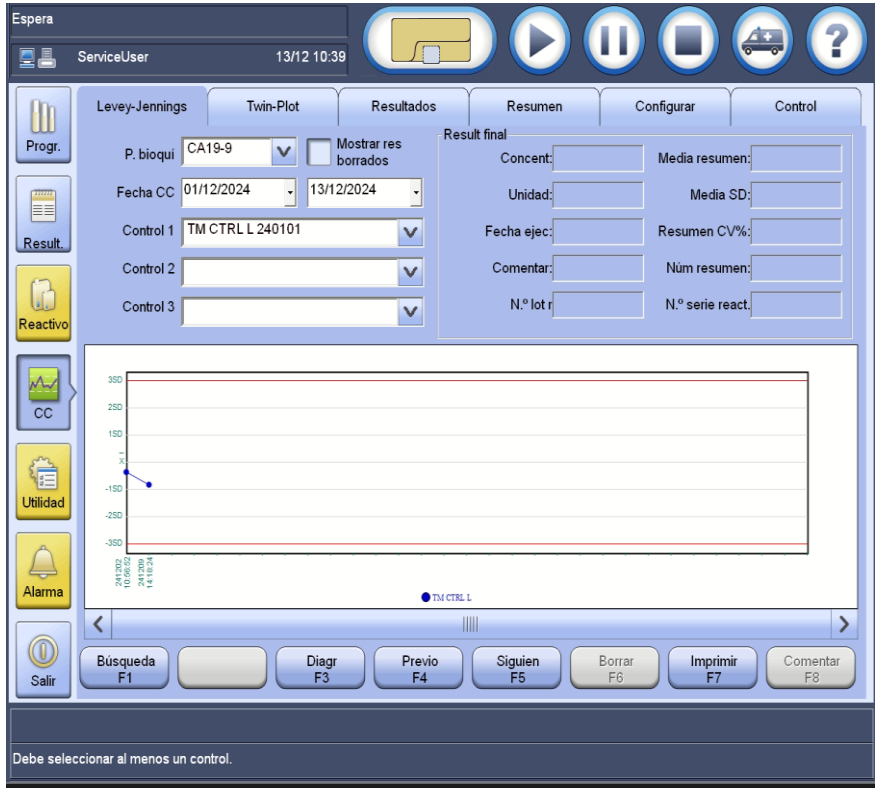
Nivel	Concent	RLU	Indicad.	N.º lote sustrato	N.º serie sustrato	Fech/hora calib
C0	0	63086,118		20240101	02154	06/09/2024 14:51:35
C0	0	3542,572		20240101	02154	06/09/2024 14:52:15
C0	0	3479,276		20240101	02154	06/09/2024 14:52:55
C1	88,99	141902,823		20240101	02154	06/09/2024 14:53:35
C1	88,99	141377,365		20240101	02154	06/09/2024 14:54:15
C2	897,18	1205790,705		20240101	02154	06/09/2024 14:54:55
C2	897,18	1193361,561		20240101	02154	06/09/2024 14:55:35

Case Ideas

Troubleshooting process:

1.3. Check QC results





QC under control

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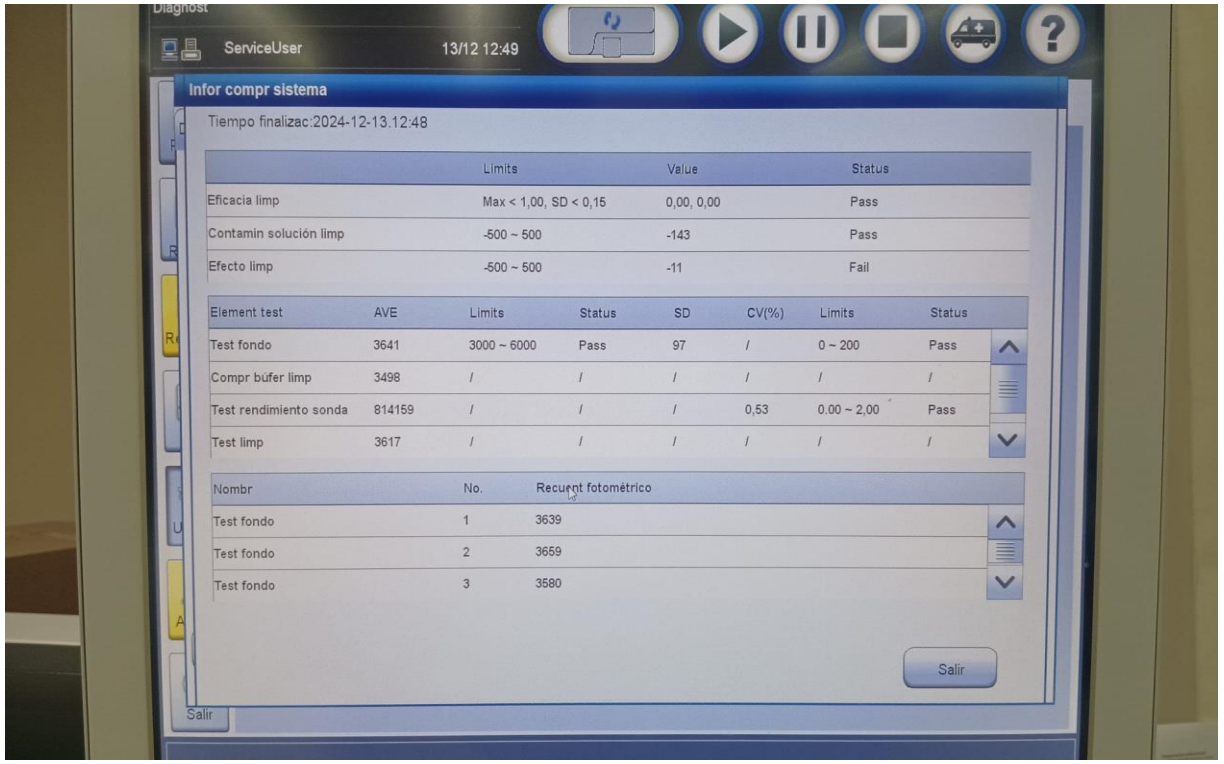
Case Ideas

Troubleshooting process:

1.4. Check system maintenance was performed



Maintenance successfully done

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Case Ideas

Troubleshooting process:

2. Try to remove sample interferences (if any)

WHY?

- Different sample interferences (such as the consumption of medications - hormone therapy, drugs, endogenous interference , etc.-, contrast agents…) can affect the results

HOW?

- Perform *PEG & HBT* anti-interference tests
- Freeze and properly store (<20°C) the patient's sample while the PEG and HBT special tubes were purchased and delivered by HQ

Case Ideas

Troubleshooting process:

2. Try to remove sample interferences (if any)

PEG

- **Principle :** PEG precipitates proteins by steric hindrance. PEG 6000 has been reported to completely precipitate immunoglobulin M (IgM) and IgG, as well as up to 80% IgA.
- **SOP:** Patient serum is mixed with twice the volume of PEG, incubated at 37 ° C for 1 hour, then centrifuged at 15,000g for 10 minutes. If the results of patients were sharply decreased , no significant change was observed in control serum. This suggests that endogenous disruptors in patients' serum may come from immunoglobulins.

HBT （Heterophile Blocking Tube/Reagent）

- **Principle :** HBT usually contains animal-derived IgG (e.g., bovine IgG, mouse IgG), inert proteins (e.g., BSA), or other blocking agents. The animal IgG in HBT is similar in structure to the animal antibody in the detection reagent, and can preferentially bind to the heterophile antibody in the sample and occupy its binding site. Inert proteins in HBT (such as BSA) or excess animal IgG can "saturate" the binding sites of heterophile antibodies in the sample, making it unable to react with animal antibodies in the assay.
- **SOP:** 250µl serum was added to HBT, and incubated at 18-28℃ for 1 hour.

Case Ideas

Troubleshooting process:

2. PEG & HBT anti-interference tests performance

CA125

Reagent lot.: 240101
Cal. lot: 230901
QC lot: 240101

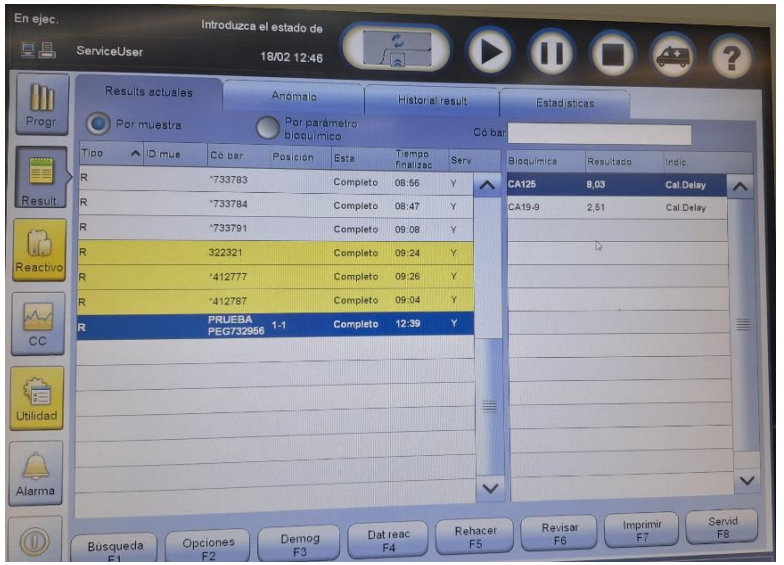
CA19-9

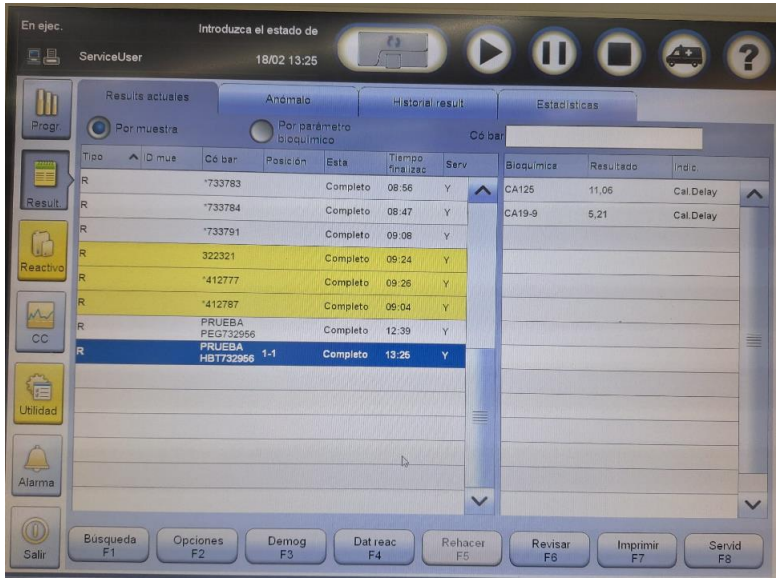
Reagent lot: 240301
Cal. lot: 230601
QC lot: 240101

1) **For PEG tube:**
   - **CA125:** 8.03
   - **CA19-9:** 2.51

2) **For HBT tube:**
   - **CA125:** 11.06
   - **CA19-9:** 5.21

Still both CA125 & CA19-9 negatives for cancer





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Case Ideas

Troubleshooting process:

3. Method comparison

We were able to collect more useful information on this case:

- Patient's **cancer type:** *breast*
- After receiving the false negative results from our Mindray CL-1200I analyzer, the patient decided to make **further tests** in another laboratory (with an immunoassay instrument from **R** brand) around January 2025; which turned out *negative* as well

Case Solution

Troubleshooting process:

3. Method comparison

HOW?

- Customer takes some number of samples (10~20) to send for analysis in the instruments of main competitors after performing the tests in Mindray's analyzer
- Make an internal analysis for data comparison of CA125 & CA19-9 parameters between different detection systems

Case Solution

Troubleshooting process:

4. Method comparison

WHY?

There are differences between systems in the measured values of CA125 & CA19-9 manufacturers, which makes it difficult to compare and verify results between different detection platforms, due to:

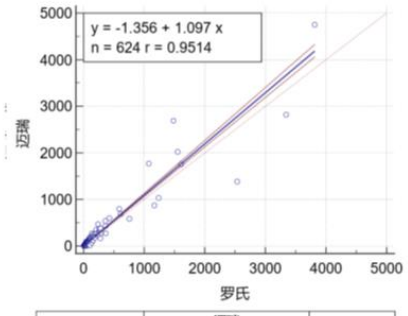
1. Immuno-traceability and standardization are not perfect yet [1]
2. Heterogeneity of immune antigens [2]
3. Differences between technology platforms, such as marker selection and performance, analytical instrument characteristics, etc. [2]

References
[1] Zhang Jiangtao, Zhang Chuanbao, Ma Rong, et alInterlaboratory quality evaluation and method comparability analysis of tumor marker testing in China[J].Chin J Medical Journal, 2010, 90(14):5
[2] Chinese Medical Association Laboratory Branch, Clinical Laboratory Center of the Ministry of Health, Editorial Committee of Chinese Journal of Laboratory MedicineSuggestions for the clinical application of tumor markers[J].Chinese Journal of Laboratory Medicine,2012,35(2):103-116

NOTE: we could not perform the comparison analysis because the reference lab denied to provide their analyzer brand. However, we have shown tests and data provided by HQ to the customer, who have accepted.

E.g.: CA125 Mindray vs Roche

Overall coincidence rate was 97% → good



符合率统计		迈瑞		符合率
		阳性	阴性	
迈瑞	阳性	140	16	89.7%
	阴性	2	468	99.6%
总符合率				97.1%

序号	迈瑞	罗氏	贝克曼	一致性	
参考范围	<35	0-35	0-35	迈瑞vs罗氏	贝克曼vs罗氏
615	25.58	74.60	24.60	不一致	不一致
626	31.85	38.00	31.50	不一致	不一致
305	15.76	104.60	16.40	不一致	不一致
344	23.55	42.00	25.70	不一致	不一致
648	20.55	38.20	21.50	不一致	不一致
654	24.80	51.90	19.70	不一致	不一致
659	18.97	48.60	16.30	不一致	不一致
664	9.41	45.00	9.60	不一致	不一致
666	22.63	44.20	22.70	不一致	不一致
671	16.56	91.40	19.30	不一致	不一致
678	17.54	36.70	19.40	不一致	不一致
53	42.56	23.22	31.50	不一致	阴性
64	26.85	58.48	28.70	不一致	不一致
187	32.66	43.75	28.30	不一致	不一致
383	33.39	36.53	28.50	不一致	不一致
385	11.75	48.65	8.40	不一致	不一致
78	35.62	23.66	32.60	不一致	阴性
690	32.55	38.80	35.50	不一致	阳性

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Case Solution

Troubleshooting process:

4. Method comparison

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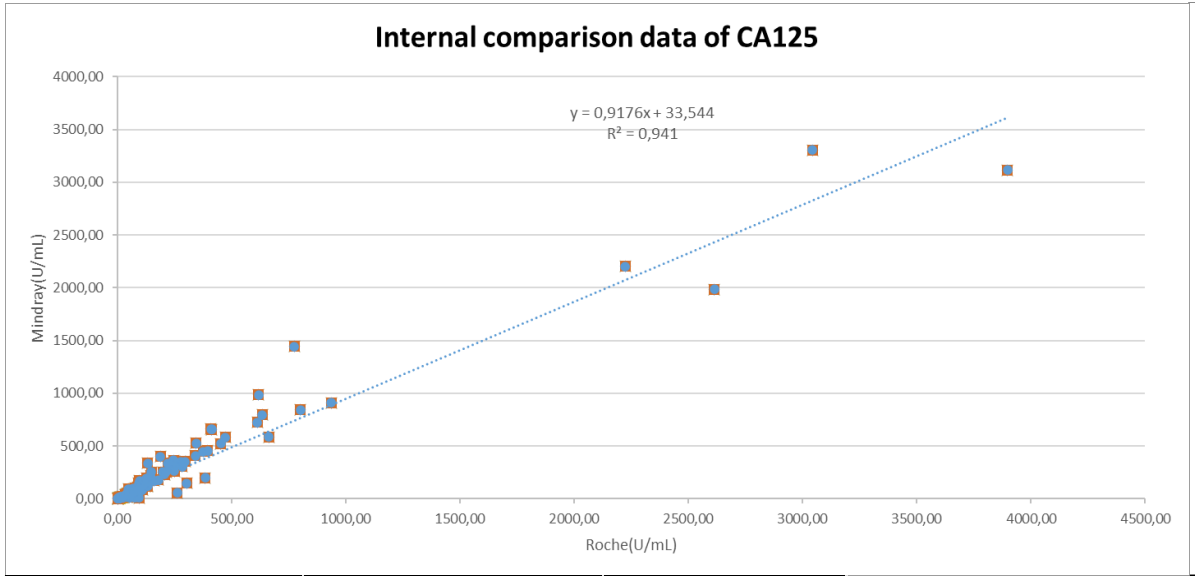
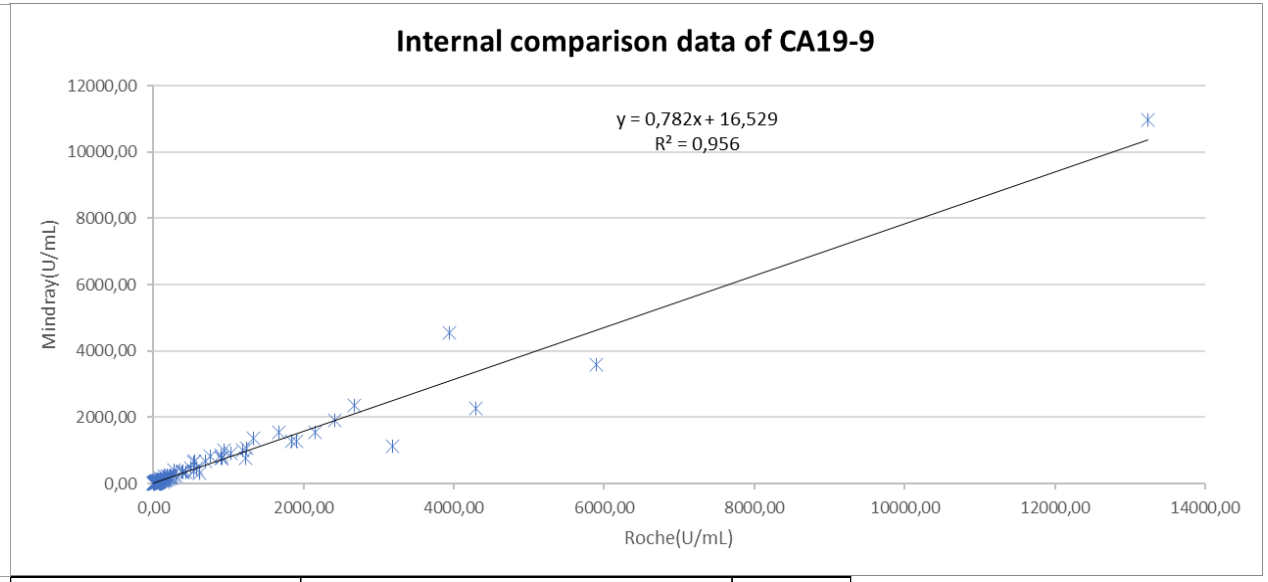


Item		R		Total
		Negative	Positve	
Mindray	Negative	57	11	68
	Positive	5	127	132
Total		62	138	200
Negative concordance rate		91,9%		
Positive concordance rate		92,0%		
Total concordance rate		92,00%		



Item		R		Total
		Negative	Positve	
Mindray	Negative	30	5	35
	Positive	2	103	105
Total		32	108	140
Negative concordance rate		93,8%		
Positive concordance rate		95,4%		
Total concordance rate		95,00%		

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Case Solution

Troubleshooting process:

4. Clinical interpretation

WHY?

There are differences between systems in the measured values of CA125 & CA19-9 manufacturers, which makes it difficult to compare and verify results between different detection platforms, due to:

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[2] Chinese Medical Association Laboratory Branch, Clinical Laboratory Center of the Ministry of Health, Editorial Committee of Chinese Journal of Laboratory MedicineSuggestions for the clinical application of tumor markers[J].Chinese Journal of Laboratory Medicine,2012,35(2):103-116

Case Summary

Conclusions :

- If there are samples that are inconsistent with the test results of Mindray and other platforms, you can determine whether it is an abnormal sample according to the following suggestions for negative results: Judging from the data values of the patient's tumor marker examination, clinical diagnosis and medication/treatment:
  - ✓ If the patient is diagnosed with a cancer type related to a certain tumor marker and the specificity of it is high for this cancer type (e.g.: ovarian cancer/CA125)
  - ✓ If the patient has not received any treatment and has shown strong positives for multiple tumor markers, consider using other systems/original machines for retesting or further information about the clinical situation
  - ✓ If necessary, consider further serial dilution, PEG precipitation or HBT incubation experiments to confirm the presence (or not) of sample interferences

Case Summary

Conclusions:

- *Tumor markers are not gold standards* for cancer diagnosis. They should be comprehensively analyzed with other clinical results, such as imaging and histopathological examination
- The patient's *clinical history* and cancer type may affect results
- The *specificity and sensitivity* of a single tumor marker are limited and thus, it is recommended to combine multiple tumor markers for detection
- In the *same patient* before and after treatment and follow-up, the *same detection system/method/reagent* should be used for testing for the same tumor marker as much as possible
- In the same patient, if the detection system/method/reagent of tumor markers needs to be changed, the patient's baseline level should be re-established if it is necessary to change the detection system/method/reagent

References
[3] Niu Aijun, Du Lutao, Jing Xu, et al Application status and suggestions of tumor markers in different detection systems[J].J Clin Laboratory Works, 2021, 39(3):4
[4] Yan Feng Recommendations for the clinical application of tumor markers[C]. Symposium on Research Progress and Application Specifications of Tumor MarkersChinese Medical Association.2012
[5] EP12-A User Protocol for Evaluation of Qualitative Test Performance. NCCLS. 22(14).

Thanks!

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