The Analysis of the Ferritin HOOK Problem

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Case Background

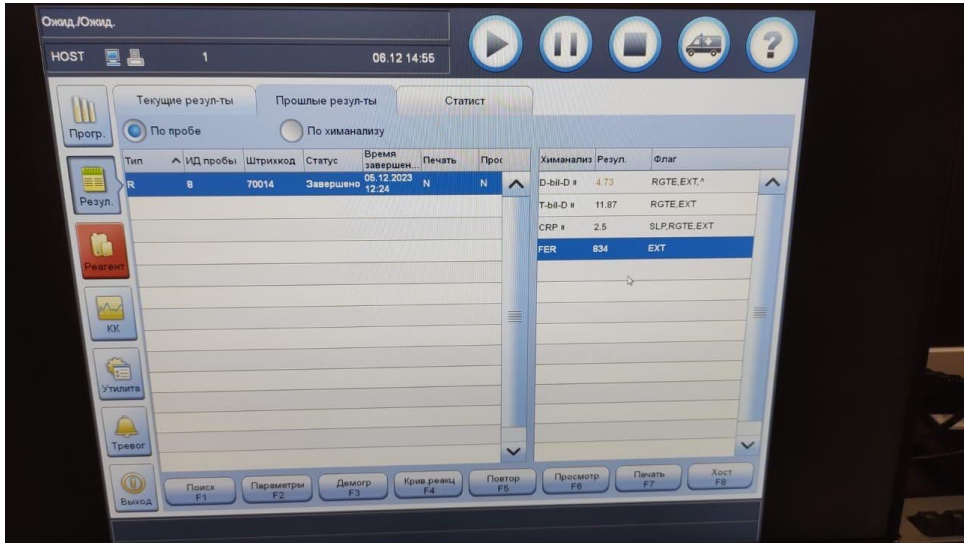
- A sample from a thalassemia patient (D56) tested inaccurate for ferritin on the BS-480 instrument, and there was no any flag.

- Testing Results

Times	Mindray	Roche	Dilution
First	834 ng/ml	8000 ng/ml	1
Second	5980 ng/ml	8200 ng/ml	10

Mindray ref range (IVDD): Male:30-400 ng/mL Female:15-150 ng/mL
Linearity Range: 10 ~ 1000 ng/mL

- The customer feedback that the patient's previous result was 9505 ng/mL and it was a D56 patient, and the Mindray initial test result was inconsistent with the actual situation.
- The results of Mindray were proved wrong by Roche retest.
- There is no alarm for abnormal results on BS-480;



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Case Ideas

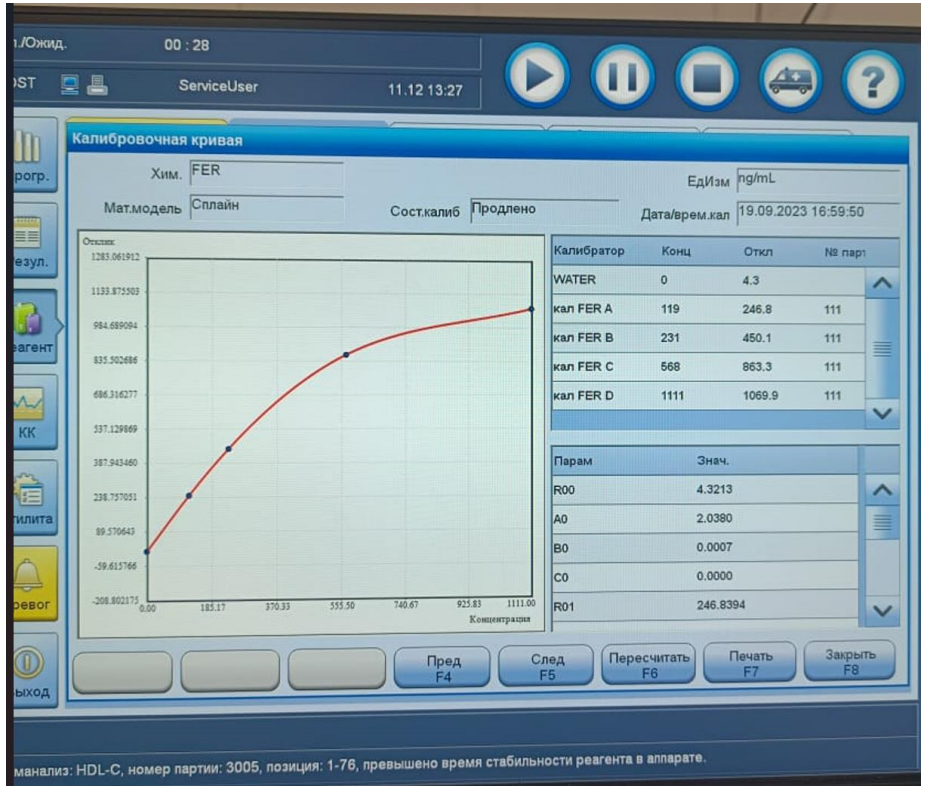
Analysis ideas:

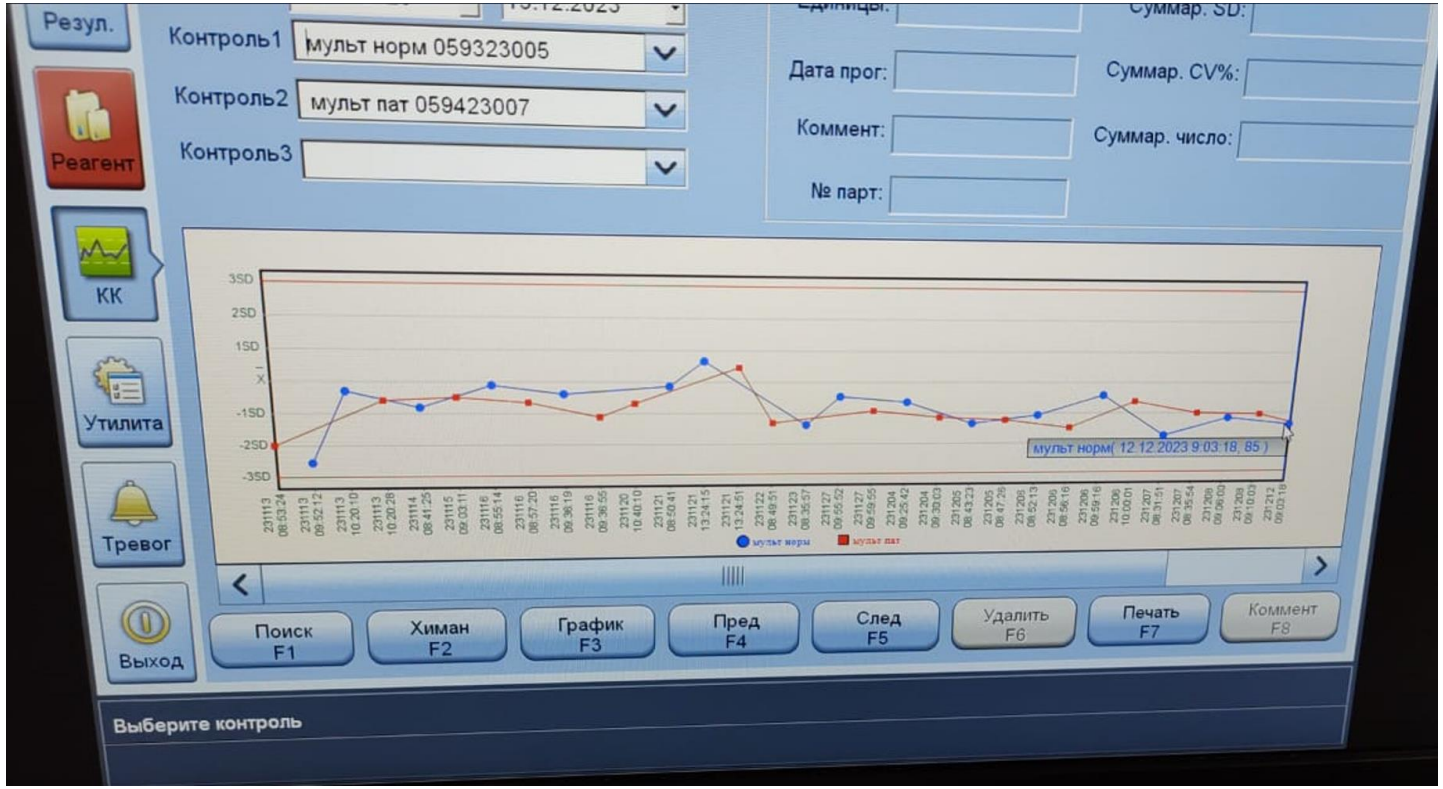
- Check the status of instruments and samples to confirm the accuracy of test results;
- Find out why the results are abnormal;
- Find the reason why the instrument did not provide an abnormal result flag;

Case Solution

Instrument status check:

✓ The quality control results were under control, and the overall quality control trend was observed. The overall quality control results were low, with a deviation of **less than 10%.**





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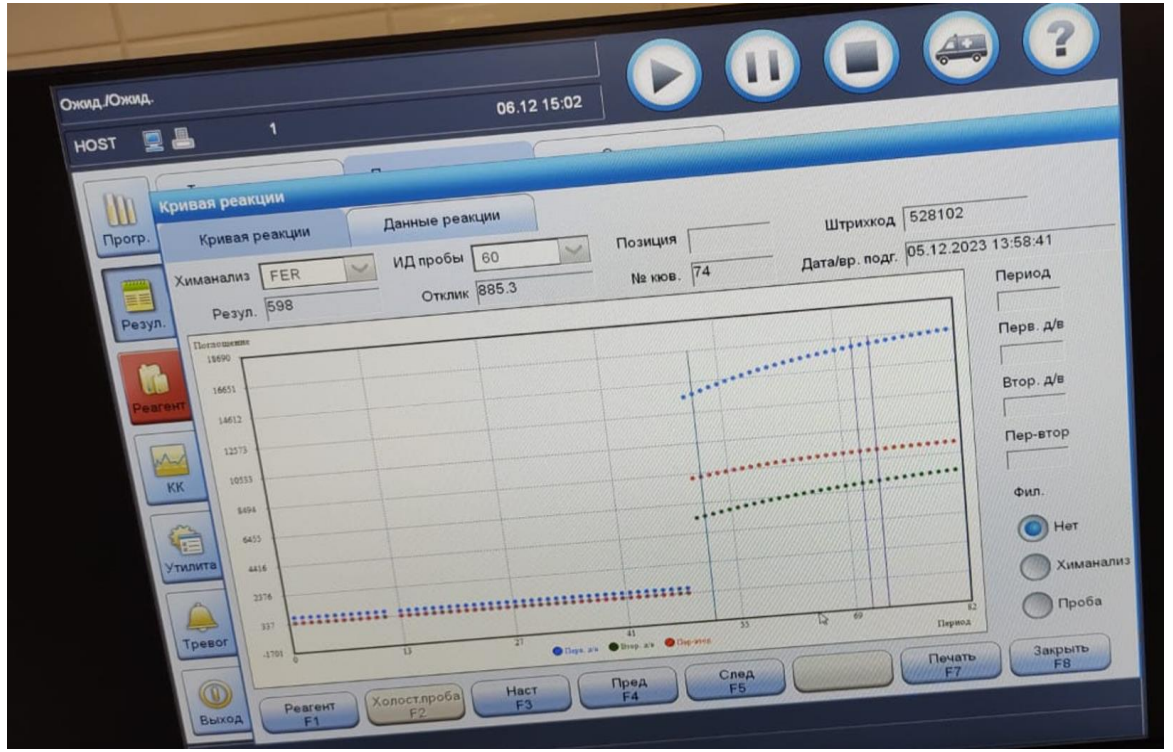
Case Solution

Reaction curve check:

- ✓ The two test results showed that the curves were smooth, and the elevation of the second wave was similar to the factory calibration reaction curve, without obvious abnormalities.



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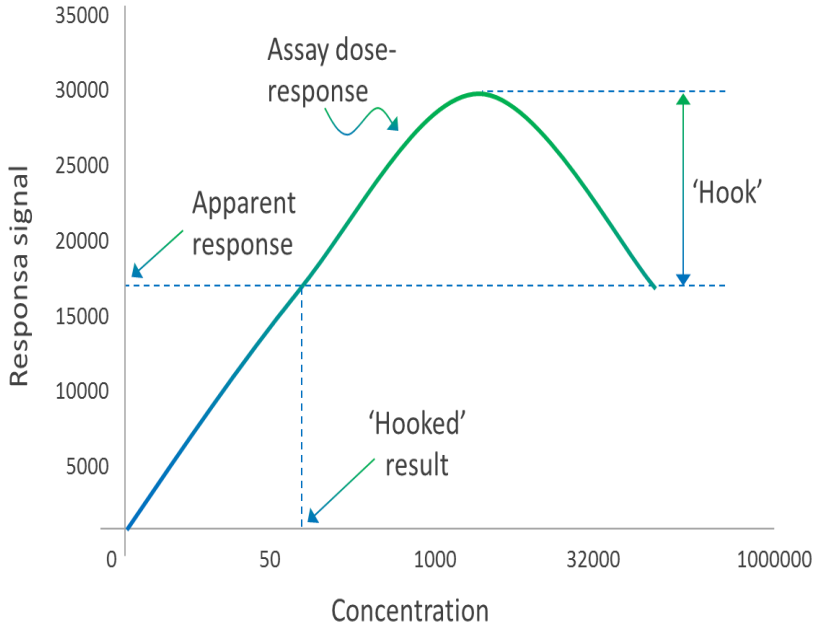
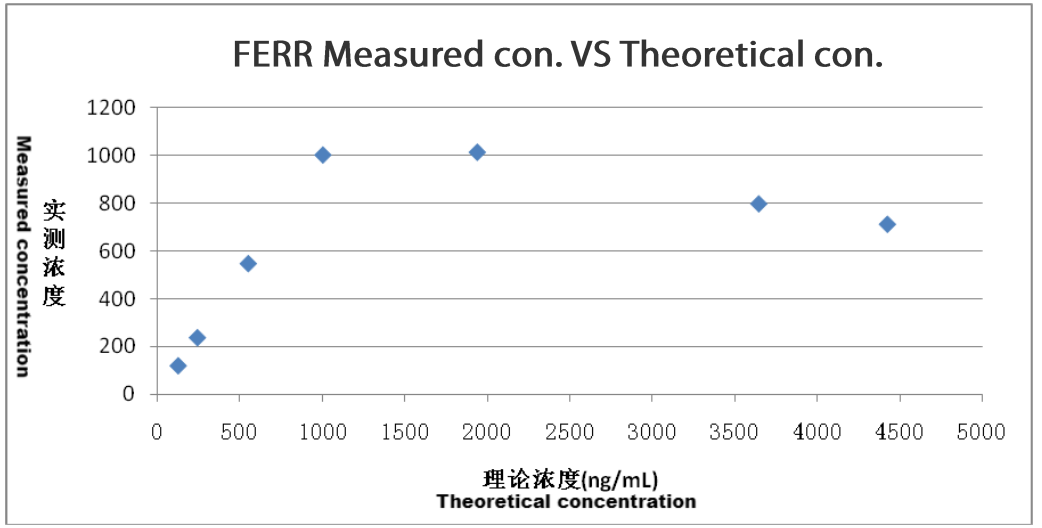
Case Solution

Why is the test result low and abnormal?

- The basic principle of immunoturbidimetry is that when the antigen and antibody reaction ratio is appropriate, the soluble complex formed will precipitate under the action of the polymerizing agent in the dilution system, forming microparticles and making the reaction solution turbidimetric. When the antibody concentration was fixed, the amount of complex formed increased with the amount of antigen in the sample, and with it the turbidity of the reaction solution. By measuring the turbidity of the reaction solution and comparing it with a series of standards, the antigen content in the sample can be calculated.
- In the reaction of antigen-antibody, the insoluble antigen-antibody complex is closely related to the ratio of antigen-antibody. At the appropriate ratio, the insoluble antigen-antibody complex is the largest, the light transmittance is the least, and the absorbance is the largest. "Above and below this ratio, the amount of insoluble antigen-antibody complexes formed decreases, the light transmitted increases, and the absorbance decreases.





- According to the test principle of FER, combining the historical results and the results of Roche, there was a HOOK effect in this FER test.

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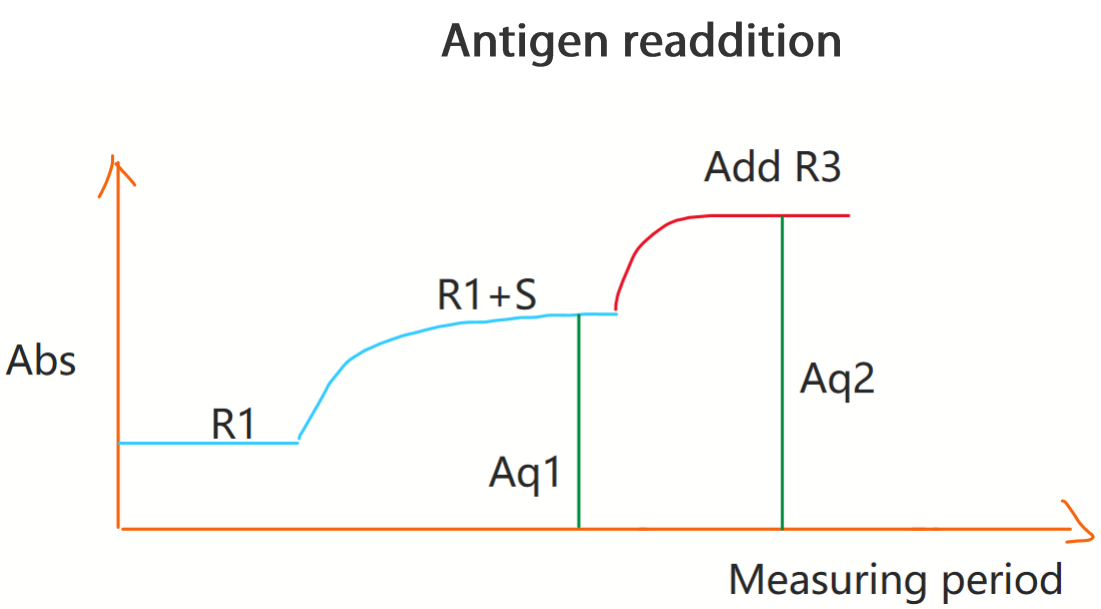
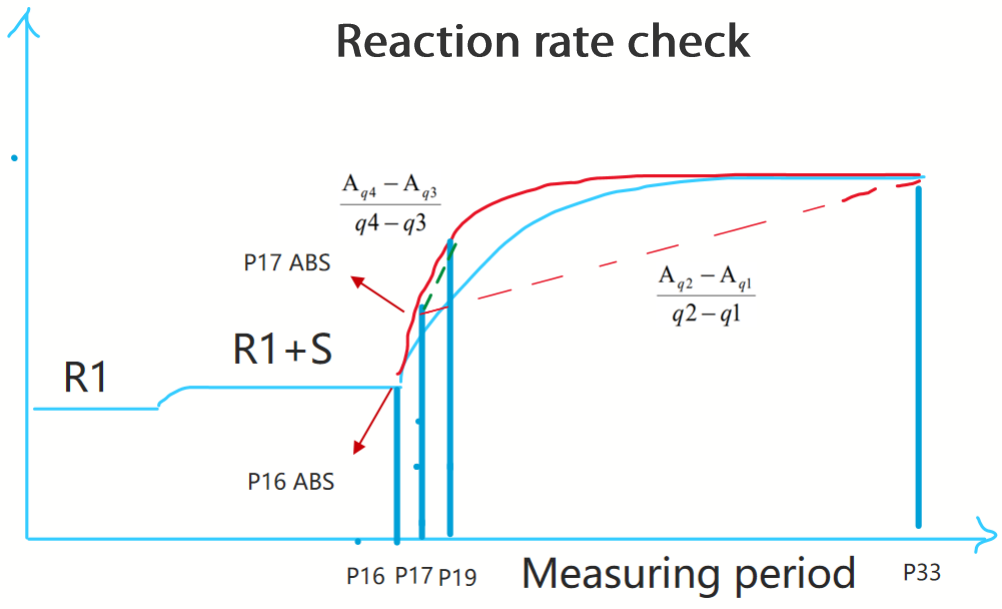
Case Solution

Why did BS-480 not show abnormal results?

- The prozone function marks the abnormal result, and the result is marked as "PRO", which prompts the user to deal with or automatically retest.
- There are two kinds of prozone tests: antigen readdition and reaction rate check.





- Under normal circumstances, if the sample concentration is high, the system will check the prozone, and the sample result will appear "PRO" alarm.
- BS-480 has no prozone function, while Mindray **BS-600M, BS-800M, BS-2000M, and BS-2800M** have this function, so the instrument does not have abnormal results alarm;
- *BS-480/490 has been discontinued;*

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Case Summary

Summary:

- In general, thalassemia is a chronic hemolytic anemia, the content of ferritin is relatively high, this kind of samples can be diluted in advance;
- If the manufacturer can provide the prozone parameters, this function can be turned on;
- If customer encounter abnormal results, they can use another biochemical instrument, such as BS-800, or test on a Mindray CLIA machine for FER.

High Dose Hook

For the CL-series FERR assay, no high dose hook effect was observed when samples containing up to approximately 80,000 ng/mL of FERR were assayed.

Case Extension

- **What biochemical tests do HOOK effects occur?**

Turbidimetric	Colorimetric
CysC, β2-MG, RBP, mALB, ApoA1, ApoB, Lp(a), FR-CRP, MYO, D-Dimer, cTnI, PA, FER, TRF, ASO, RF, CRP, C3, C4, IgA, IgG, IgM, IgE	*UA, UREA, CREA, TPUC, TC, TG, HDL-C, LDL-C, CK, CK-MB, LDH, α-HBDH, ACE, HCY, ALT, AST, γ-GT, ALP, T-Bil-V, DBil, TBil, TBA, TP, ALB, CHE, ADA, 5'-NT, AFU, Fe, UIBC, G6PD, Glu, HbA1c, FUN*

- **Which Mindray biochemical assay have prozone check function?**

Mindray Prozone Assay	Flag
MALB, RFⅢ, FER, MYO, PA	PRO

Mindray Prozone Assay	Flag
ALP, r-GT, a-HBDH, CK-MB, LDH LIP, CO2, CHE	BOE

➢ Some enzyme assays use a prozone check function to indicate substrate depletion.

Case Extension

Solutions to the HOOK effect

1. **Optimize the antibody content;** Excessive antibody was added into the reagent;

➢ A literature showed that the higher the concentration of IgM antibody in the IGM reagent kit, the later the hook effect appeared, and the wider the safety report range.

2. **Optimize the detection method**, Mindray particle-enhanced detection technology;

➢ Mindray particle-enhanced technology uses multi-titer antibody microspheres to improve the reaction sensitivity and detection range.

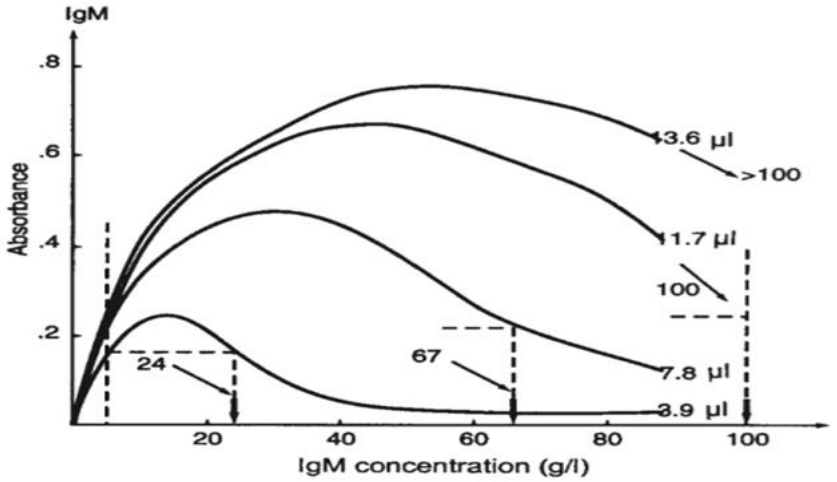
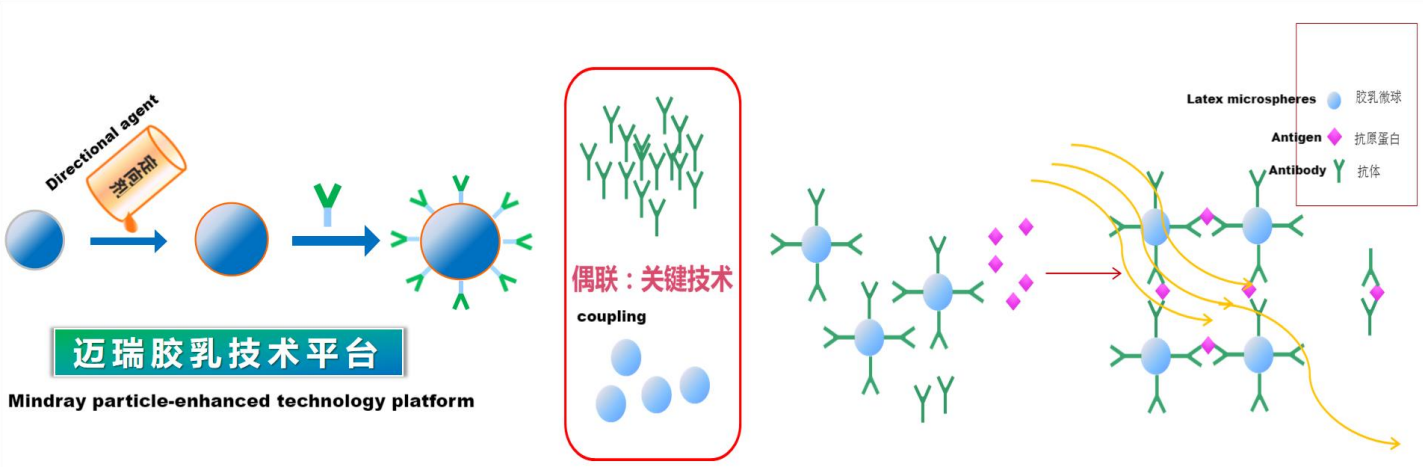


Fig. 20 Dose-response curves for IgM at four different antibody concentrations; 3.9 µl–13.6 µl are volumes of antibody; 24, 67, and 100 are critical points.



Ref:
1.Clin Chem Lab Med 2001; 39(11):1098-1109

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Case Extension

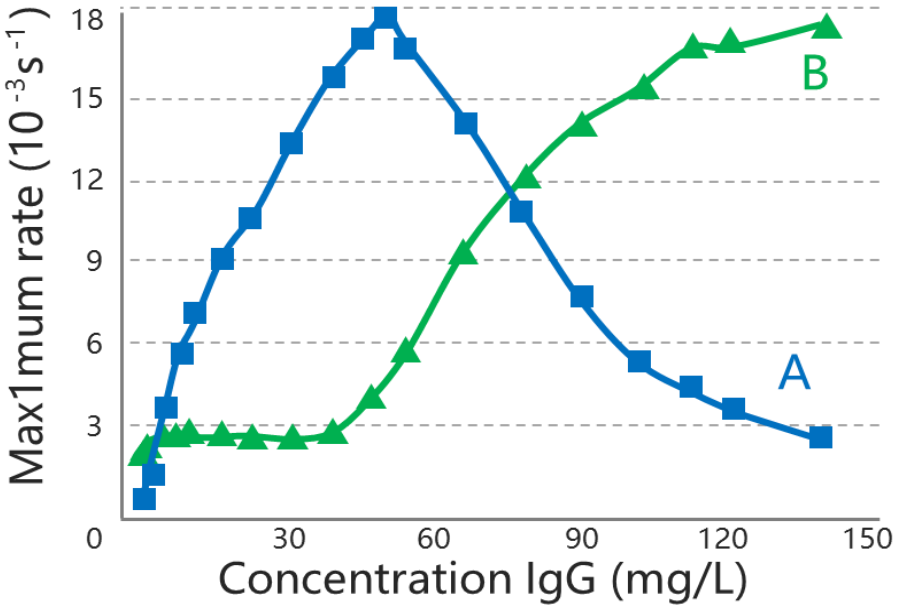
Solutions to the HOOK effect

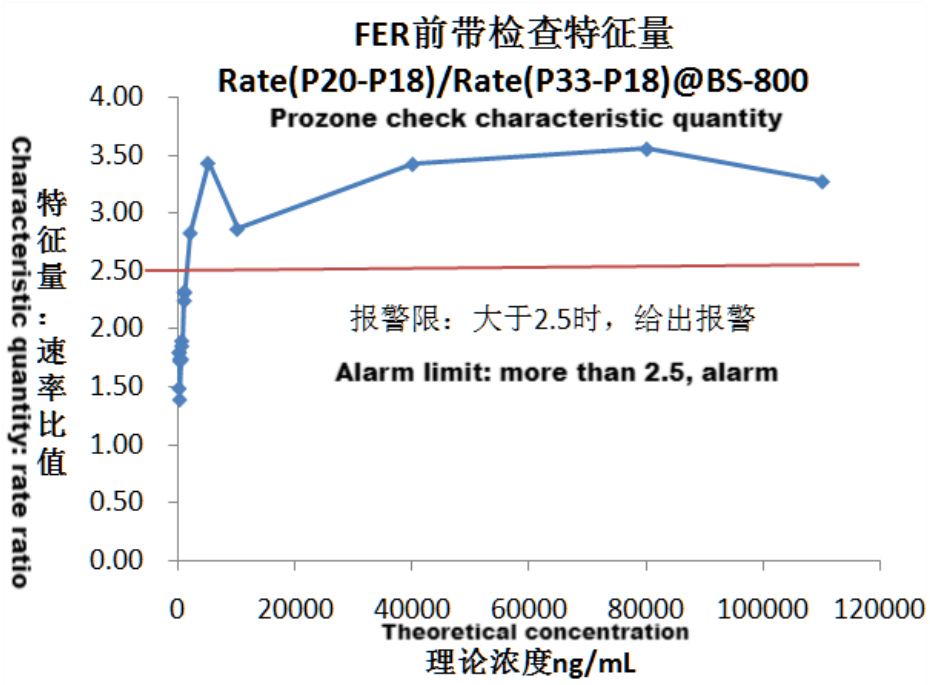
3. Establish a mathematical model to determine antigen excess;

- Some studies have shown that excess antibody, equivalent antigen and excess antigen can be distinguished according to the kinetic characteristics of antigen-antibody reaction.
- Use Mindray's ProZone parameters;

4. Eliminate sub-wavelength to improve HOOK concentration;

- Single-wavelength detection is used to improve the reactivity. This method needs the support of the manufacturer's software, and need to verify the quality control target.





Ref:
1.Kinetic turbidimetric method for the immunochemical quantification of immunoglobulins, including samples with excess antigen. Clin Chem. 1988 Feb;34(2):309-15

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Case Extension

Solutions to the HOOK effect

5. Sample dilution

- The samples beyond the linear range were diluted or automatically diluted. If the diluted results were still out of the range, the test was continued to increase the dilution factor, and the sample was comprehensively analyzed combined with the patient's clinical information.
- Use a specific protein analyzer;

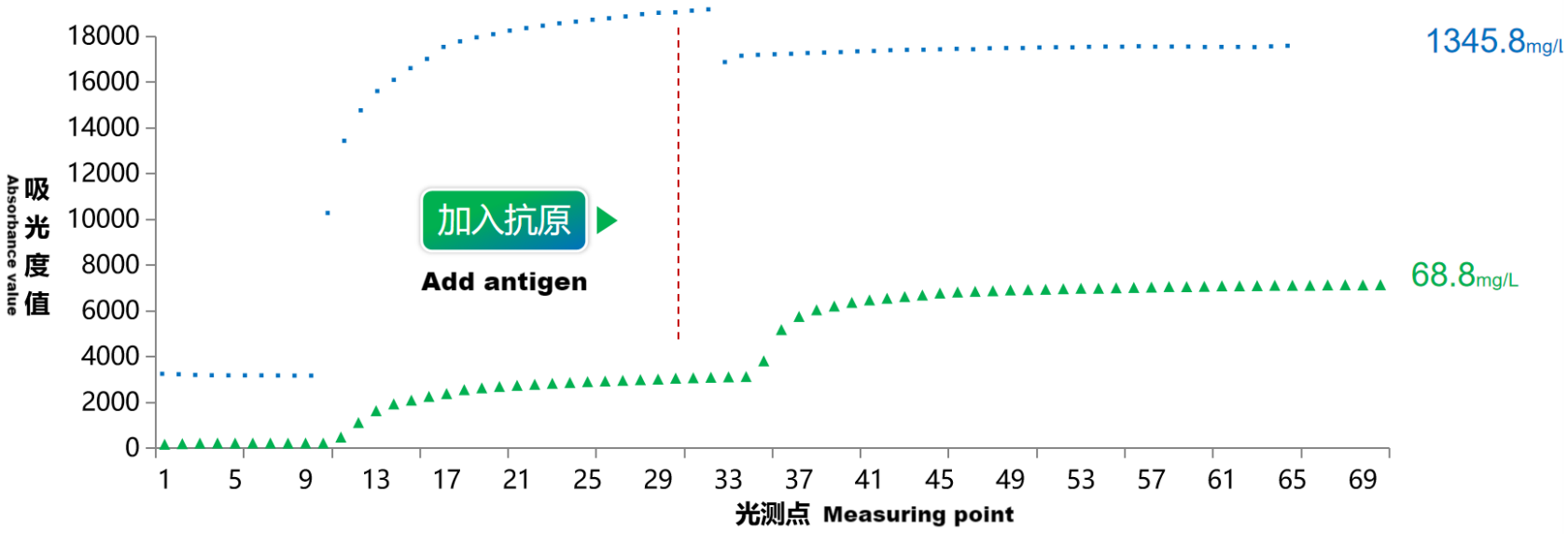
Assay	Unit	Hook			Solution		
		Mindray	A company	B company	Mindray	A company	B company
IgA	g/L	25	100	100	Prozone	21 times pre-dilution	Prozone
IgM	g/L	50	100	100	Prozone	21 times pre-dilution	Prozone
IgG	g/L	>350	400	300	/	21 times pre-dilution	Prozone
C3	g/L	>22	12.5	/	/	21 times pre-dilution	/
C4	g/L	>9	5	/	/	21 times pre-dilution	/
RF	IU/mL	>13000	6000	1500	/	Prozone	Prozone
ASO	IU/mL	>19200	4000	/	/	21 times pre-dilution	/
CRP	mg/L	>600	1200	750	/	Prozone	Prozone
mALB	mg/L	>21780	40000	6000	Prozone	Ag readdition	/
ApoA1	g/L	>13.9	11	/	/	21 times pre-dilution	/
ApoB	g/L	>11	9	/	/	21 times pre-dilution	/
Lp(a)	g/L	>3700	1900	/	/	/	/
PA	g/L	>2665	2500	/	/	10 times pre-dilution	/
FER	ng/mL	2000	80000	20000	Prozone	Prozone	Prozone
D-Dimer	μg/mL	>420	220μg FEU/L	200μg FEU/L	/	/	Prozone
MYO	ng/mL	>98544	15000	20000	/	/	/
TRF	g/L	12	17	/	/	21 times pre-dilution	/
CysC	mg/L	>100	20	/	/	/	/
β2-MG	mg/L	>500	/	200	/	/	/

Case Extension

Solutions to the HOOK effect

6. Antigens readdition

- The antigen was added again, and if there was a reaction indicating excess antibody, the result was reliable.
- If there is no reaction or decrease, it indicates excessive antigen and the result is suspicious.



MALB readdition reaction curve

- This method will increase the cost of reagents, and the test process is complex and inefficient.

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Thanks!

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