Folates test failure

IVD International Clinical Application Department

Dorofeyev Gennadiy CAS IVD NSK

06.05.2024

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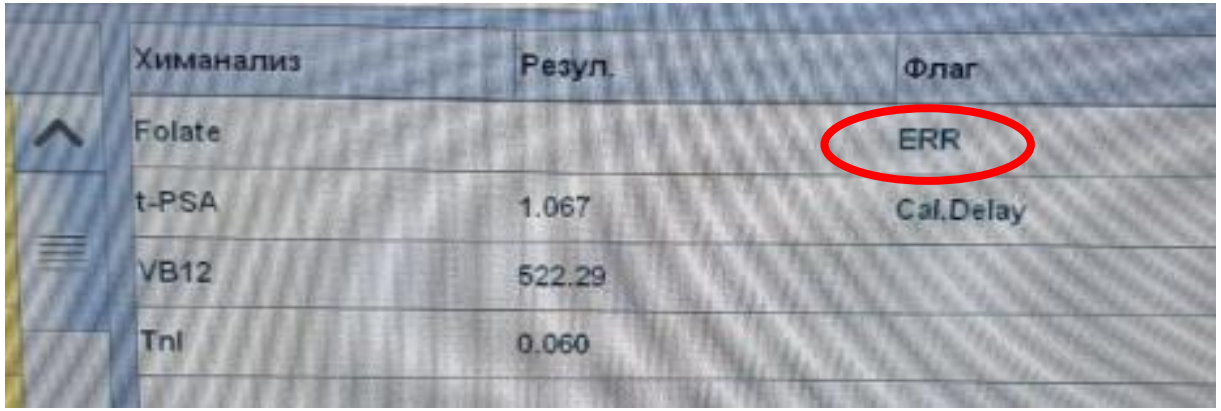
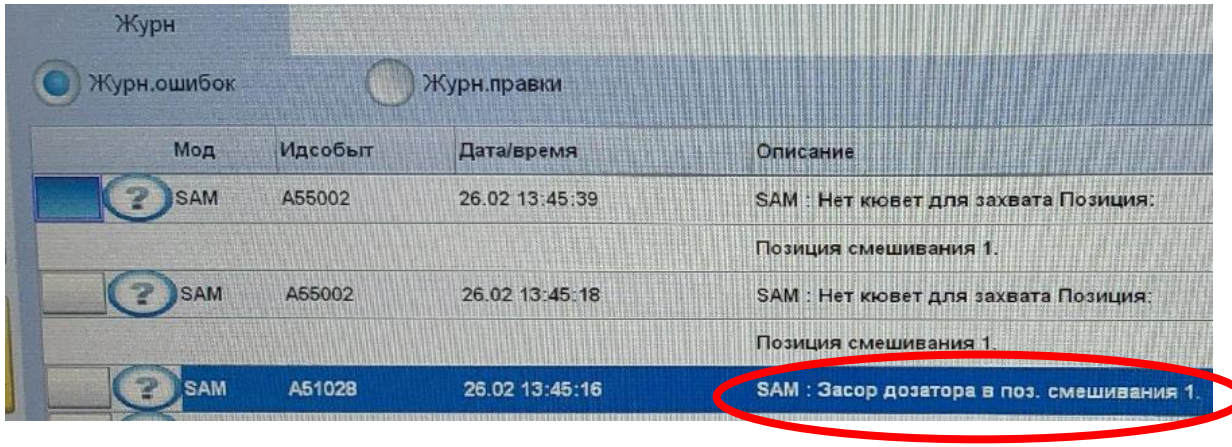
Case Background

Require:

- Folate test fails in one patient from different tubes;
- Tubes with silica, centrifuged 3000 rpm 10 minutes 30 minutes after collection;
- Other tests (t-PSA, VB 12, TnI) are performed normally;
- For folates, the **ERR flag** is displayed, in the error description **“dispenser clogged in mixing position 1”;**
- This case happened in **city clinical hospital №25, Novosibirsk.** A large laboratory in which all lines of Mindray analyzers are installed, and the first installation of a coagulation analyzer in Siberia is also performed.

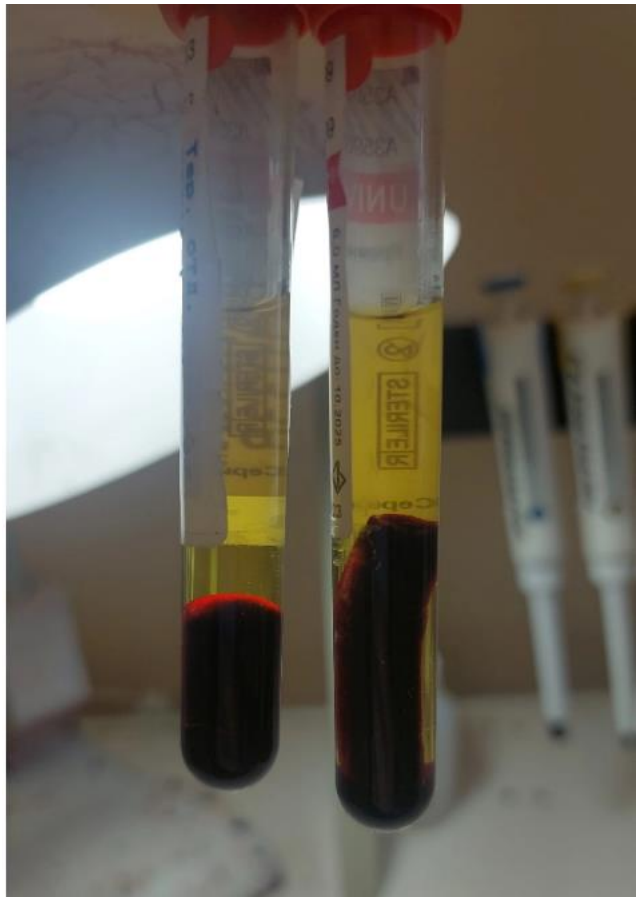




Case Ideas

Follow :

- Ask are there any problem with folate tests in other patient's samples;(no problems)
- Ask about daily/weekly/monthly maintenance; (regular maintenance)
- Ask about same problems on other tests; (no problems)
- Ask about preanalytical rules, time from sample collection to testing, sample storage conditions; (everything is fine)
- Ask about patient's anamnesis and previous test results;
- Check the sample status. (regular sample)



Case Ideas

Mind process:

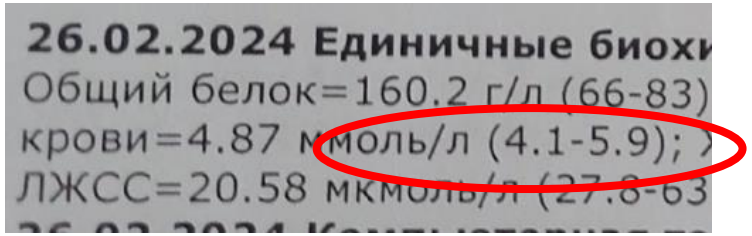
- They don't have problems with folate test in other samples, they do maintenance correctly, and they don't meet with the same problem on other tests. This lab performs only in-patient samples. Estimated time from blood collection to analysis is 4 hours. They sent me a photo of the test tube, it has no visual abnormalities;
- Since the other tests from this tube were performed correctly, then the reason may be due to pretreatment reagent because if the probe was initially unable to take a sample due to the clot after first incubation, then none of the tests from this tube would have been performed, but f-PSA, VB-12 and TnI are okay;
- I suggested that doctors manually repeat the actions that the analyzer does step by step according to SOP to check what happens to the sample after adding reagents for pretreatment and incubation.

Case Solution

Patients' results:

- Patient diagnosis is «**bilateral polysegmental pneumonia**», since it is an inflammatory disease, inflammatory diseases often lead to an increase TP level in the blood.

Chemianalysis	Result	Reference range
ALT	38.2 U/L	To 50
AST	**79.2 U/L**	To 50
T. Bil	15.8 umol/L	5-21
Cholesterol	1.3 umol/L	To 5.2
Crea	**186.3 umol/L**	72-127
Gluc	4.9 mmol/L	4.1-5.9
TP	160.2 g/L	66-83
Urea	**7.54 mmol/L**	2.8-7.2
Na	**119 mmol/L**	136-146
K	**3.45 mmol/L**	3.5-5.1



Case Solution

Experiment results:

- Taking information from the SOP, doctors manually repeated the necessary steps (Sample 80uL+Pretreatment R1 50uL+Pretreatment R2 50uL and incubation for 12.5 min 37 °C).
- The sample turned into a jelly-like substance;
- I elicited the question of why a patient's sample might thicken and clog the needle. I requested the patient's history and other test results, find out very high TP results (160.2 g/L, reference range 66-83), other results were normal or not too high;
- I look up for some information to explain may the high TP reacts with the pretreatment liquid, **find that** if the concentration of total protein in the sample is high, it will form a jelly-like substance with NaOH in the pretreatment solution;

1. Manar, Abdalrazeq., C., Valeria, L., Giosafatto., Marilena, Esposito., Maria, Fenderico., Prospero, Di, Pierro., Raffaele, Porta. (2019). Glycerol-Plasticized Films Obtained from Whey Proteins Denatured at Alkaline pH. THE Coatings, doi: 10.3390/COATINGS9050322
2. Nirnay, Samanta., Debasish, Das, Mahanta., Rajib, Kumar, Mitra. (2014). Collective hydration dynamics of guanidinium chloride solutions and its possible role in protein denaturation: a terahertz spectroscopic study.. Physical Chemistry Chemical Physics, doi: 10.1039/C4CP03273J

Case Solution

Jelly-like structure mechanism:

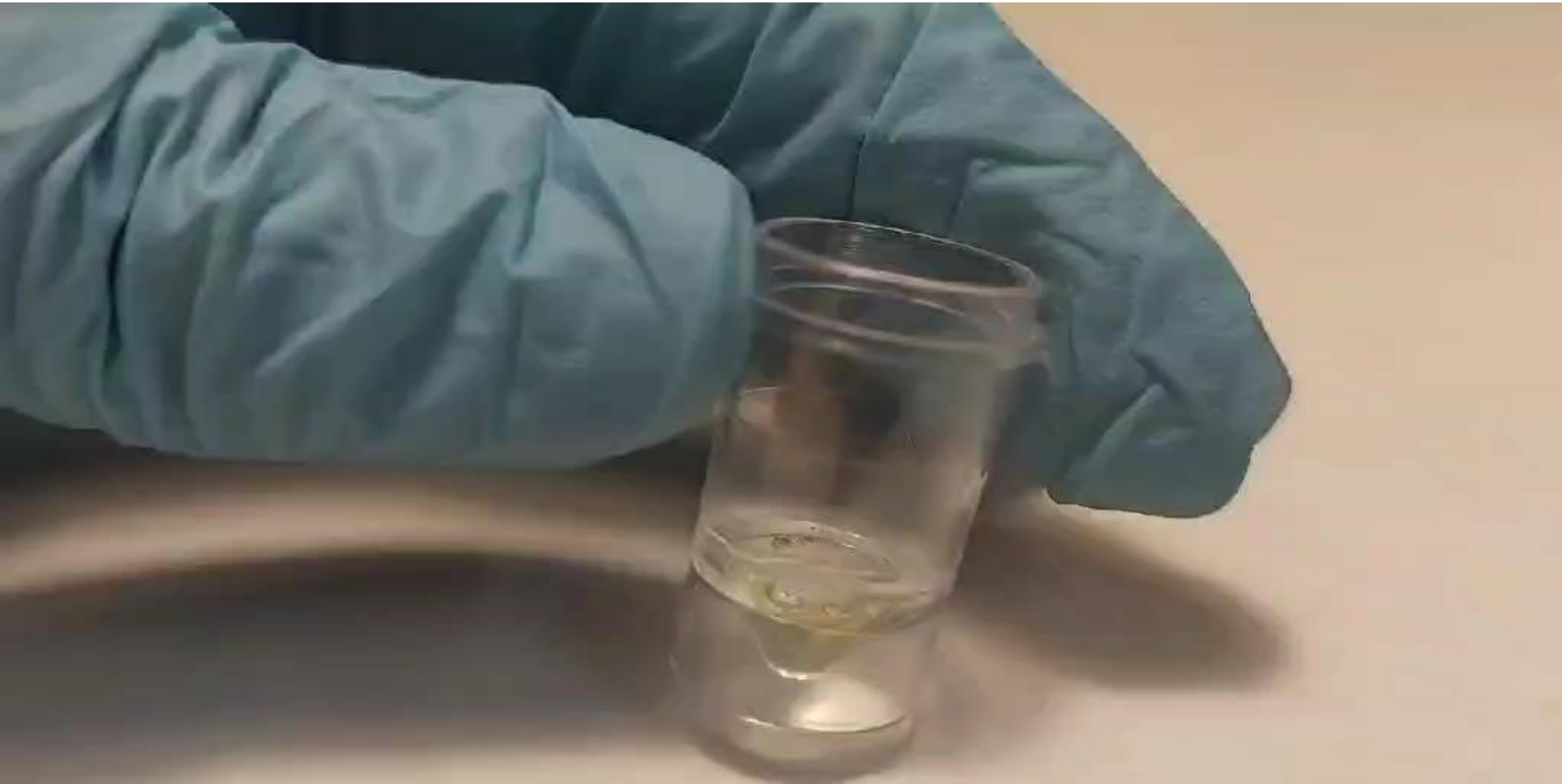
- Proteins can be denatured by NaOH due to the high alkaline conditions it creates. The denaturation process involves the disruption of the protein's native structure, leading to loss of its functional properties. NaOH can cause denaturation by several mechanisms:

1) *One mechanism involves the disruption of salt bridges that stabilize the folded conformation of proteins;*
2) *Another mechanism involves the use of NaOH in combination with other factors, such as heat treatment or the presence of crowding agents, to induce denaturation.*

- Additionally, NaOH can induce denaturation by altering the pH of the protein environment, which can affect the protein's stability and conformation. Overall, the high alkalinity of NaOH can disrupt the interactions and forces that maintain the protein's native structure, leading to denaturation;
- **Conclusion:** High TP in patient's sample can react with NaOH and it will lead to form a jelly-like substance.

1. Heleen, Meuzelaar., Matthijs, R., Panman., Sander, Woutersen. (2015). Guanidinium-induced denaturation by breaking of salt bridges. Angewandte Chemie, doi: 10.1002/ANIE.201508601
2. J., Nathan, Scott., Nathaniel, V., Nucci., Jane, M., Vanderkooi. (2008). Changes in Water Structure Induced by the Guanidinium Cation and Implications for Protein Denaturation. Journal of Physical Chemistry A, doi: 10.1021/JP8058239

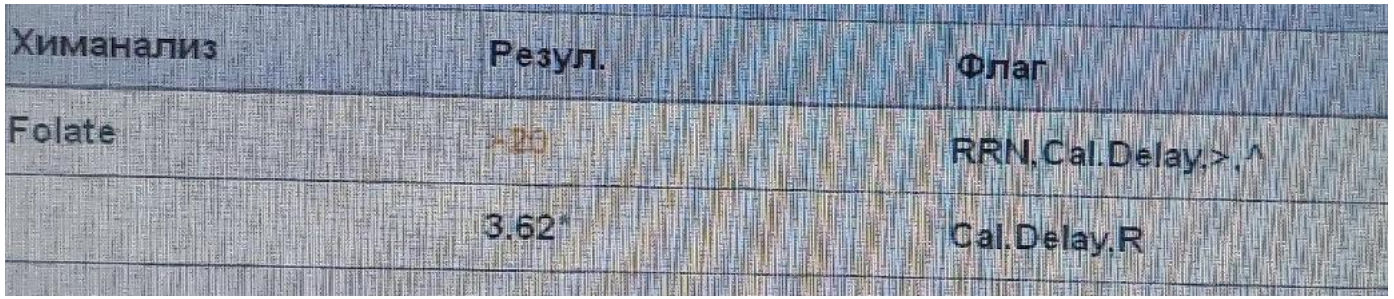
Case Solution



Case Solution

The final cause and solution to the problem:

- To obtain the Folate test results I told doctors to manually dilute sample 1:1 with C0 calibrator;
- The doctors received the results “>20” with 1:1 dilution and “3.62” with 1:2 dilution;
- I explained that maximum dilution rate for Folates is 1;1. If the dilution rate is greater than 1:1, then linearity is lost, so the “3.62” result is wrong, right result is “>20”.

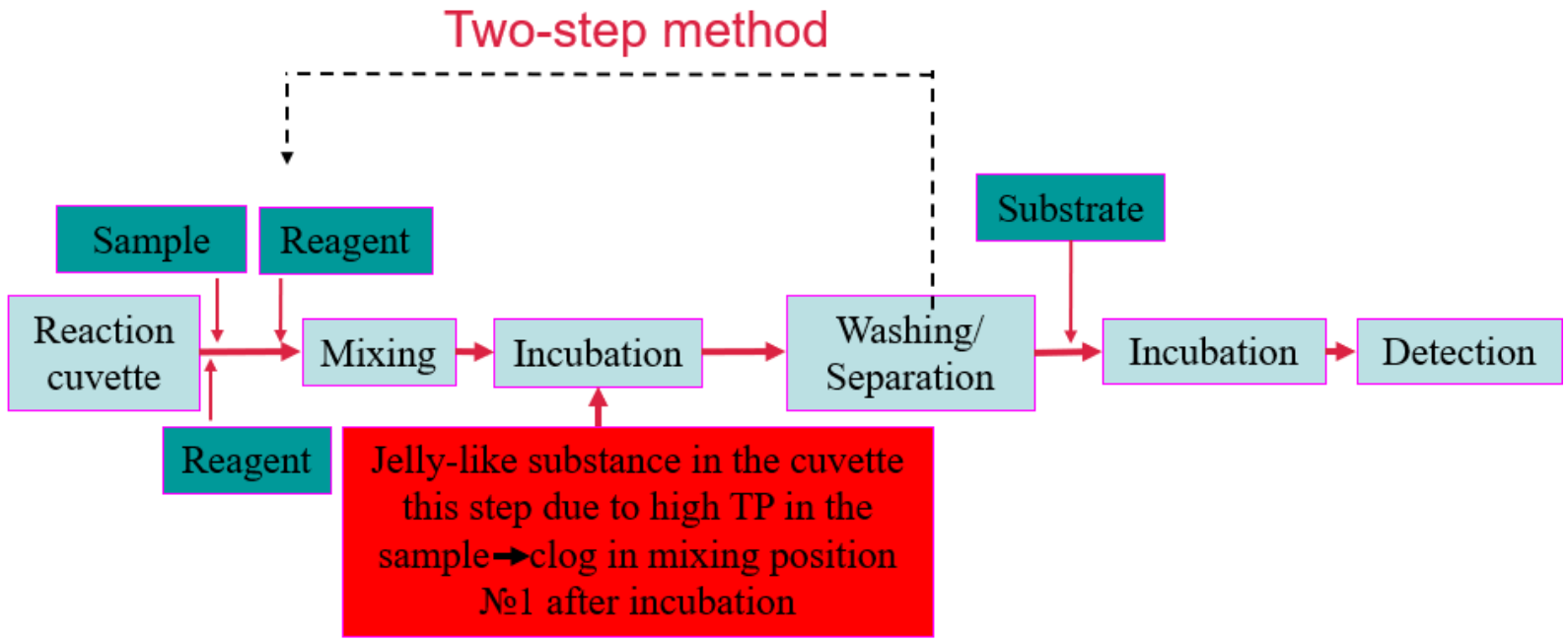


Case Summary

Require:

- Helped doctors get a valid result in a short time;
- Found out the cause of such an error by conducting an experiment;
- Found the mechanism of formation of jelly-like substance;

Reaction principle and case explain:



Recommendations for same issues:

1. Check other samples for same problem, check analyser and reagents status;
2. Check preanalytical step, samples storage conditions, turn around time;
3. Check patient anamnesis and other test results, if you find abnormal results look at the composition of the reagent, and search the articles for information on the interaction of abnormal result with the components of the reagent;
4. Dilute the sample to get valid result.

Thanks!

mindray 迈瑞