Real Time Stability Study of Simple Simon PT Reagent, product number ZAF-101-2

Intended use of the study results and of the study report document.

Study design

Two sets of five lots of PT reagents were secured for the study. The lots were manufactured 2, 3, 4, 7 and 9 years ago and have since then been stored refrigerated at 2℃ to 8℃ in closed original flasks; each lot being composed of one flask of freeze dried biologicals and one flask of dissolution buffer of the same lot as the biologicals. One such set of the five lots of PT reagent was removed from the refrigerated storage and placed at 37℃ for seven (7) days, after which the flasks were returned to refrigerated storage. At a later point in time, one to four weeks later, closed flasks of reagent and corresponding buffer were, one by one, opened and the buffer in the buffer flask poured into the flask with the freeze dried biologicals, as described in the instructions for use. The properties of the thus reconstituted PT reagent were, after being kept for two to four hours at room temperature, investigated by use to determine the INR of an abnormal control plasma (ZAP lot U384L), and also investigated for the size of the "signal" that followed the clot occurrence.

Experimental details

The five (5) different lots of PT reagent, and their date of manufacture in parenthesis, were; N223M (week 22, 2013), P161M (week 16, 2015), S182L (week 18, 2018), T032L (week 3, 2019) and U041L (week 4, 2020).

Each of the ten varieties of reagent (the five lots of SSPT reagens where each is either exposed or not exposed to 37℃ for 7 days) were used to repeatedly, twelve times, determine the INR of the ZAP. In all 120 determinations, 60 on one day with reagent not exposed to 37℃, and 60 determinations six days later with reagent exposed to 37℃. Prior to the determination by one lot of the reagents, e.g. N223M, the reagent specific settings of the reader, including the calibration parameters, were adjusted to those employed when the reagent lot in question was in clinical use, e.g. as for lot N223M was the case 9 years ago.The reader specific settings were the same throughout the study. All the study's 120 determinations were thus performed on one and the same reader, but with the reagent specific settings identical to those used with a given lot reagent when this lot was in clinical use.

In the study, the 10 uL of sample (i.e. ZAP) was added to and mixed with the 200 uL of reagent using a 10 uL pipette. Mixxocaps were not used in the study but were checked to yield identical results within 2%.

The ZAP used in the study, lot U384L, expiration date September 2023, is supplied by Zafena with reference INR levels in the range 2,30 to 2,80 (INR 2.55 +/- 0.25).

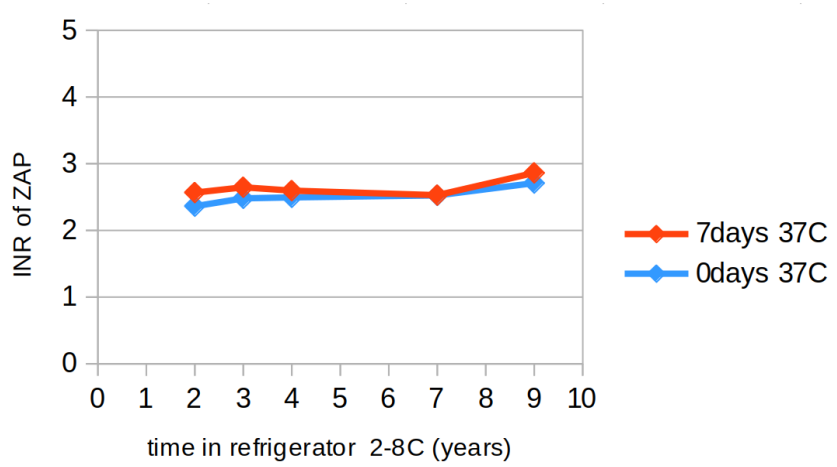
The temperature in the refrigerators used to store the closed flasks of SSPT reagent were specified to hold a temperature of 2℃ - 8℃. This temperature was checked periodically and was typically in the range 2℃ - 4℃. The refrigerators were fitted with a beep alarm should the temperature be outside of the range 2℃ to 8℃. The alarm on the refrigerator in which the reagents in question were stored was triggered less frequently than once a year, and never for a duration of more than one day.

Results and discussion

As stated above, the INR of the ZAP was determined twelve times with each of the 10 reagents, to view all data see appendices 1 and 2. The CV of 12 determinations was in the range 1.4% to 3.1%, with a mean of 2.0%. Mean INR of the ZAP was 2.51 with reagents not exposed to 37℃ for 7 days and 2.64 with reagents so exposed.

Lot PT reagent	U041L	T032L	S182L	P161M	N223M	Mean
Time 2-8C (years)	2	3	4	7	9	**5.00**
0 days 37C	2.36	2.48	2.50	2.53	2.71	**2.51**
CV, n=12	2.0%	2.3%	2.3%	3.1%	1.9%	**2.3%**
7 days 37C	2.57	2.65	2.60	2.53	2.86	**2.64**
CV, n=12	1.8%	2.1%	1.4%	2.0%	1.4%	**1.8%**
Mean INR	2.46	2.56	2.55	2.53	2.79	**2.58**

Below is the above data, but in a graphic display:

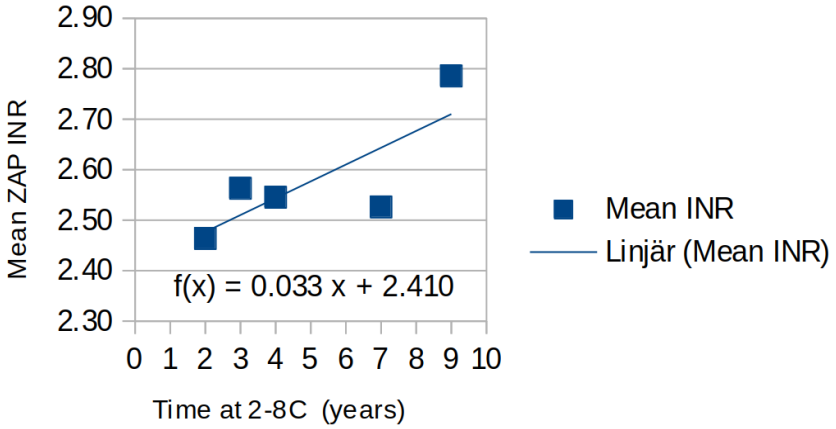


In the study, SSPT reagents stored refrigerated for 3 to 7 years displayed no obvious differences when used to determine the INR of an abnormal control plasma (ZAP lot U384L), the mean of INR was 2.50 +/- 0.09% (CV). With reagent stored refrigerated for two years, the INR was 5.6% lower and with reagent stored refrigerated for 9 years the INR was 8.4% higher. With reagents that, in addition to the refrigerated storage, were stored 7 days at 37C, the corresponding values were 2.57 +/- 2.3% (CV), 0.8% lower and 10.4% higher, respectively..

The question, difficult to answer, is, are the observed differences in reagent properties, at least in part, due to changes induced by storage, or were the differences present all-ready at the time of

manufacture. Some part of the difference must surely be due to differences in the calibration to which the systems were subjected at the time when the SSPT lot in question was taken into clinical use. A piece of evidence that supports the notion that differences in reagent properties are induced by storage, is the fact that longer storage has more effect, as is true for increased storage temperature.

The graph below, Graph 2, indicates that the INR of an abnormal control (ZAP lot U0384L) increases when analyzed with a reagent that, in closed flasks, has been subjected to increased storage time at refrigerator temperature.



Graph 2 supports the notion that reagent stored refrigerated for a progressively long time period yields increasingly high INR when used to determine the INR of (ZAP lot U384L). The increase is estimated at 0.033 INR units per year of storage at refrigerator temperature..

Appendix 2 data indicates that, in addition to year-long refrigerated storage, 7 days storage at 37°C, in closed flasks, also shows increased INR when the reagent is used to determine the INR of abnormal control plasma. The average observed INR increase caused by the 7 days storage at 37°C was 0.13 INR units or 3.9 fold more than the 0.033 INR units caused by one year of refrigerated storage.

An interesting result of the study is thus that 7 days exposure to 37°C affects the SSPT reagent as much as 3.9 years of storage at refrigerated temperature. In short; one week at 37°C has as much effect on a SSPT reagent as four years in the refrigerator.

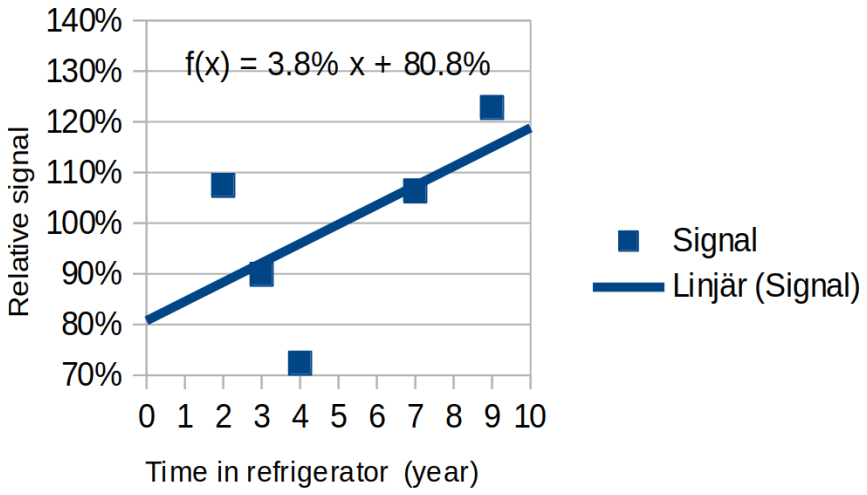
Decline in the "clotting signal" as a measure of reagent instability

Those who work with the SSPT system know that the "clotting signal", a measure of the rapidity of the clot formation, is a property of the reagent, assumed to be coagulation factor V dependent, that deteriorates with time. The signal observed when the INR of ZAP lot U384L was determined with the 5 here studied reagents, each with or without a seven days exposure at 37℃, is given in the table below:

Lot SSPT reagent	U041L	T032L	S182L	P161M	N223M	Mean
Time at 2 – 8C (years)	2	3	4	7	9	**5**
Signal at 0 days at 37C	260	220	170	280	300	**246**
Signal at 7 days at 37C	230	190	160	205	260	**209**
Mean signal	**245**	**205**	**165**	**243**	**280**	
Signal reduction by 7 days 37C	-12%	-14%	-6%	-27%	-13%	**-15%**

The table high-lights the fact that 7 days exposure to 37℃ decreases the signal by 15%, on average.

Graph 3 below displays the mean signal of the determinations with each of the five lots of reagent plotted against the storage time at refrigerator temperature:



In this study, 9 years of storage at refrigerator temperature has no detectable effect on the clotting signal. In fact, the signal appears to increase with time of refrigerator storage, which is unreasonable. The differences observed are likely to have been present at the various times of manufacture.
THE RESULTS OF THIS REAL TIME STABILITY STUDY WARRANTS THE SHELF-LIFE OF THE SSPT REAGENT ZAF 101-2 TO BE INCREASED FROM THREE (3) YEARS TO FIVE (5) YEARS.

APPENDIX 1: Five lots of Simple Simon PT reagent stored 2 to 9 years, in closed flasks, at 2℃ - 8℃. On a given day in August 2022, the closed flasks, one lot at a time, were removed from the refrigerator and content of the buffer flask was added to the flask with freeze dried biologicals thus "reconstituting" the reagent. The reconstituted reagent was stored for one (1) hour before being put to use in determining the INR of an abnormal control plasma, ZAP lot U384L.

Test date	2022-07-19	2022-07-19	2022-07-20	2022-07-20	2022-07-20
Operator	FW	FW	FW	FW	FW
Manufactured	**2013-05**	**2015-03**	**2018-05**	**2019-04**	**2020-03**
stored at	2-8C	2-8C	2-8C	2-8C	2-8C
stored time	**9 years**	**7 years**	**4 years**	**3 years**	**2 years**
Due date	2016-05	2018-03	2021-05	2022-04	2023-03
Reagent LOT	N223M,	P161M;	S182L'	T032L'	U041L,
ZAP LOT	U384L	U384L	U384L	U384L	U384L
ZAP reference value	2,3 -2,8	2,3 - 2,8	2,3 -2,8	2,3 -2,8	2,3 -2,8
Reader	255	255	255	255	255
Reader calibration	N223M	P161M	S182L	T032L	U041L
1	2,78	2,56	2,48	2,38	2,27
2	2,72	2,53	2,53	2,50	2,3
3	2,83	2,54	2,51	2,45	2,35
4	2,70	2,50	2,52	2,56	2,32
5	2,67	2,54	2,54	2,50	2,36
6	2,67	2,52	2,52	2,49	2,39
7	2,68	2,74	2,52	2,51	2,36
8	2,67	2,49	2,49	2,48	2,41
9	2,73	2,41	2,47	2,41	2,39
10	2,72	2,51	2,33	2,57	2,38
11	2,65	2,51	2,48	2,42	2,37
12	2,69	2,45	2,55	2,50	2,44
Mean	**2,71**	**2,53**	**2,50**	**2,48**	**2,36**
CV	1,92%	3,14%	2,32%	2,31%	2,00%

APPENDIX 2: Five lots of Simple Simon PT reagent stored 2 to 9 years, in closed flasks, at 2℃ - 8℃. On August 1, 2022, the closed flasks were moved to a heating cabinet at 37℃, kept there until August 8 (for 7 days, one week), and returned to the refrigerator. On August 15 the closed lots, one lot at a time, were removed from the refrigerator and content of the buffer flask added to the flask with freeze dried biologicals, thus “reconstituting” the reagent. The reconstituted reagent was stored for one (1) hour before being put to use in determining the INR of an abnormal control plasma, ZAP lot U384L

Test date	2022-08-08	2022-08-09	2022-08-09	2022-08-09	2022-08-09
Operator	FW	FW	FW	FW	FW
Manufactured	**2013-05**	**2015-03**	**2018-05**	**2019-04**	**2020-03**
stored at	2-8C	2-8C	2-8C	2-8C	2-8C
stored in 37C	1v	1v	1v	1v	1v
stored time	**9 years**	**7 years**	**4 years**	**3 years**	**2 years**
Due date	2016-05	2018-03	2021-05	2022-04	2023-03
Reagent LOT	N223M,	P161M;	S182L'	T032L'	U041L,
ZAP LOT	U384L	U384L	U384L	U384L	U384L
ZAP reference value	2,3 -2,8	2,3 - 2,8	2,3 -2,8	2,3 -2,8	2,3 -2,8
Reader	255	255	255	255	255
Reader calibration	N223M	P161M	S182L	T032L	U041L
1	2,87	2,42	2,60	2,63	2,57
2	2,84	2,47	2,67	2,52	2,57
3	2,81	2,51	2,60	2,67	2,52
4	2,96	2,58	2,56	2,57	2,58
5	2,83	2,56	2,65	2,67	2,63
6	2,88	2,54	2,60	2,68	2,58
7	2,82	2,54	2,59	2,66	2,56
8	2,84	2,53	2,55	2,68	2,61
9	2,87	2,54	2,58	2,68	2,49
10	2,85	2,59	2,56	2,63	2,63
11	2,89	2,58	2,57	2,72	2,56
12	2,89	2,47	2,62	2,64	2,49
Mean	**2,86**	**2,53**	**2,60**	**2,65**	**2,57**
CV	1,42%	2,05%	1,41%	2,05%	1,83%