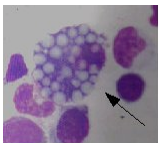
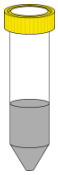
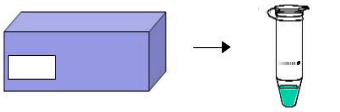
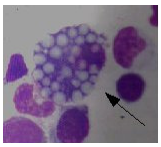


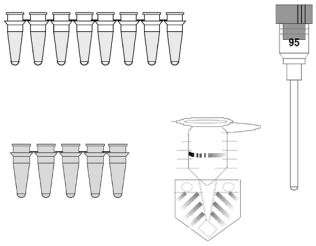
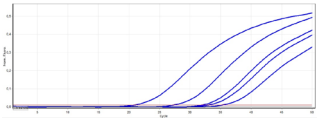
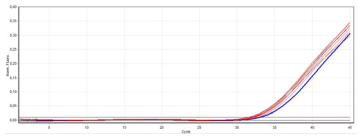
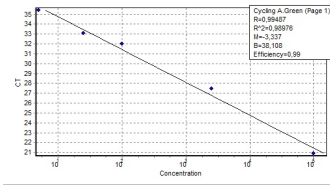
Explanation of the LIPSGENE® Gene Expression Quantification Kits

The LIPSGENE® Kits are real time PCR amplification tests for quantification of target cDNA in clinical samples. All kits include standard tubes, stably coated with given amounts of synthetic standard DNA that will be amplified in parallel with the samples. To analyse the nucleic acids in the samples, the kits contain also sample tubes or strips coated with the same amplification enhancer matrix which is borne in the standard tubes, so as to guarantee the same amplification environment for both the standards and the samples.
In order to always confirm the RNA integrity of the analyzed sample as well as to correct ~~for~~? RNA load, cDNA synthesis efficiency, PCR inhibitors and possible target loss during long-time storage, we strongly recommend to "normalize" the kit data to the number of reference genes or "housekeeping gene" transcripts, e.g. c-ABL or GAPDH transcripts measured within the same cDNA sample. For quantification of cABL or GAPDH cDNA please use respectively the LIPSGENE® c-ABL (Cat.no. 1030101LP-120, 120 tests for use with "Low profile" plastic supporting instruments) or the LIPSGENE® GAPDH Kits (e.g. Cat.no. 1030102RP-120, 120 tests for "Regular profile" plastic supporting instruments).

Perform erythrocyte lysis, wash WBC pellet with PBS buffer		15.0 ml tubes Use lysis solutions and wash buffer provided from the manufacturers of the purification kit.
Perform total RNA or mRNA extraction from WBC using the purification kit of choice		Perform extraction according to the recommendations of the manufacturer of the purification kit.
Perform cDNA synthesis of the target/ reference gene		Use the LIPSGENE® M-MLV Reverse Transcription Kit.
Prepare 5x reagent mix		Add 200 µl PCR grade water, incubate at 37°C for 10 min, mix by vortexing for 3 sec.  5 sec, 10,000 g

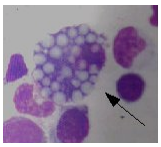
2. *LIPS*GENE® – GENE EXPRESSION QUANTIFICATION



Prepare and aliquote 1x master mix into the tubes, add sample	 Add 20 µl of 1x master mix to all sample tubes and quantification standard tubes. Add 5 µl of extracted sample to the sample tubes. Add 5 µl PCR grade water to the sample tubes which serves as NTC and to the quantitation standard tubes.  5 sec, 1,500 g
Cover tubes, set up and perform the real-time PCR run	
Analyze runs **Get target and reference gene standard and sample growth curves**	Standard growth curves  Target growth curves  Standard reference curve 
Report: qualitative or quantitative results, normalize for reference gene transcripts (e.g. GAPDH, c-ABL) quantified within the same sample	

4	**Proben "PCa-Erlangen Kollektive"**						
5							
6	Kundenangaben:			cDNA geliefert, 500 ng RNA revers transcribiert in 20 µl-Ansatz			
7	gemessener Parameter:			Survivin			
8	cDNA (Verdünnung):			1:4 (durch LIPSDIAG)			
10	Lfd. Nr.	Probenbezeichnung Intern	zmol Survivin pro 2 µl cDNA	Mittelwert	amol GAPDH pro 2 µl cDNA	Mittelwert	zmol Survivin/ amol GAPDH
11	17/17	Tu 90%	0,11642		0,05825		
12			0,11307	0,11474	0,06195	0,06010	1,90923
13	18/17	No	0,05210		0,05077		
14			0,04675	0,04942	0,05767	0,05422	0,91148
15	23/17	Tu 70%	0,10217		0,04904		
16			0,08368	0,09292	0,05688	0,05296	1,75459
17	24/17	No	0,03821		0,09335		
18			0,06189	0,05005	0,07560	0,08447	0,59249
19	25/17	Tu 100%	0,09117		0,08551		
20			0,09966	0,09541	0,10289	0,09420	1,01289
21	26/17	No	0,05908		0,05817		
22			0,05583	0,05745	0,06248	0,06033	0,95241
23	33/17	Tu 90%	0,53613		0,10117		
24			0,52658	0,53135	0,11011	0,10564	5,02980

Figure 2.1: LIPSGENE® gene expression quantification kits: Short Protocol-at-a-glance.



2.1. DETECTION / QUANTIFICATION OF MINIMAL RESIDUAL DISEASE (MRD) IN HEMATOLOGY/ONCOLOGY

Minimal residual disease (MRD) is the name given to the small number of leukemic or solid tumour cells that remain in the patient during treatment or after treatment, when the patient is in remission (no symptoms or signs of disease). It is the major cause of relapse in cancer and leukemia. Up until about two decades ago, none of the methods used to assess/detect cancer were sensitive enough to detect MRD. Now, however, very precise molecular biology tests are available - based on DNA or RNA detection and these can measure minute levels of cancer cells in body fluids, blood or bone marrow samples, sometimes at a level as low as 1 cancer cell in a million of normal cells.

In cancer treatment, particularly leukemia, MRD testing has several important roles: determining whether treatment has eradicated the cancer or whether traces remain, comparing the efficacy of different treatments, monitoring patient remission status and recurrence of the leukemia or cancer and choosing the treatment that will best meet those needs (personalization of the treatment).

References

See appendix