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Evaluation of an External Quality Assessment program for the Kleihauer test : Impact on analytical performance and patient care.

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BACKGROUND / METHOD

The Kleihauer-Betke test (KBT) is a laboratory test used to quantify foetomaternal hemorrhage. Although this method has proved to be useful clinically for the monitoring of pregnancy but also for the dose adjustment of anti-D immunoglobulin for Rhesus prophylaxis, this test is often criticized. It is due to its technical implementation and its difficulties in count and interpretation. KBT is a manual test with a high level of variability, difficult to standardize and requiring technical expertise.

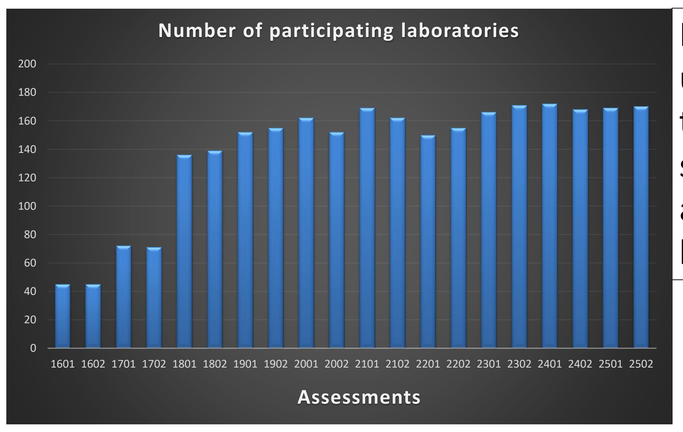
To help laboratories in standardization and interpretation of the KBT, the CNRHP and ASQUALAB has set up an EQC consisting in sending a stained smear (KHL) and a whole blood sample (KHS). Each sample is associated with a clinical case study and a clinical counseling quiz (Rhesus prophylaxis, complementary tests ...).

Since 2016, the CNRHP which integrates in its missions (ministerial circular DGOS 2004) the preparation of standard and quality control collaborated with the association ASQUALAB to set up an external evaluation of the quality. There are two assessments per year. Since 2016 twenty assessments were conducted with about 170 laboratories participating.

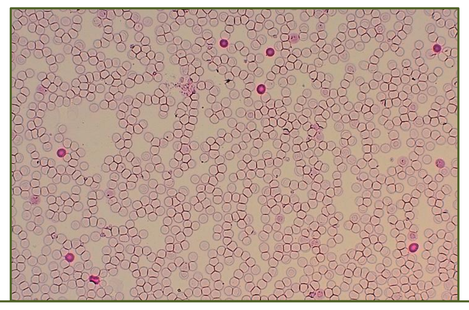
KHL and KHS come from 2 calibrated mixtures prepared from adult O RH: -1 (D-) group whole blood and O RH: 1 (D+) group cord blood. Adult whole blood RH: -1 comes from a donor collected by the French blood establishment. The negativity (absence of F-cells) of the Kleihauer test (colorimetric method and flow cytometry) was performed. The target value of each sample is obtained by flow cytometry technique with anti-D labeling (BRAD3).

A retrospective analysis of the results of all laboratories was undertaken in order to evaluate the contribution of this EEQ in the mastery of this test.

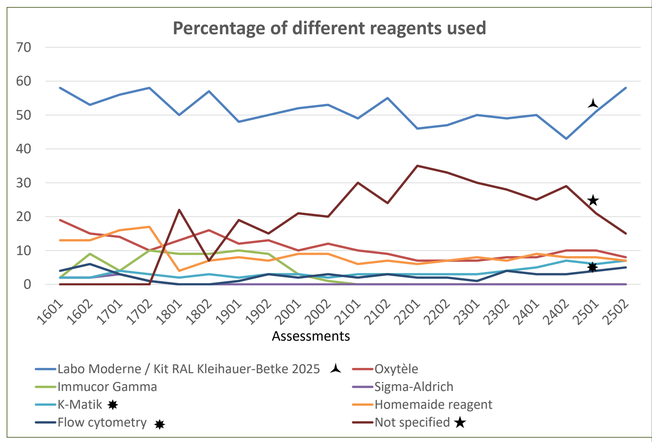
RESULTS



Increasing until 2021, then stabilizing around 170 laboratories



Example of Kleihauer-Betke test : 30 Fetal Red Blood Cells (FRBC) / 10000 Adult Red Blood Cells (ARBC)



Commercial kits are the most staining technique used.
Kit Labo Moderne (LM) +++.
LM has been marketing a new coloring reagent (2025).
The collection of the type of reagent used needs improvement. ★
Few laboratories use automated techniques. ✸

Since 2016 we have seen an improvement in the results despite a tightening in acceptable limits since 2020, more in line with the clinical management of patients and patients RH1 negative in particular. Indeed we observe a decrease in the interlaboratory variability from 30% to 20% and a reduction in the number of laboratories with results outside the acceptable limits from 50% to 25% of laboratories. This trend is more marked on KHL samples which evaluates the counting than on KHS which takes into account the entire process : smear / staining / count. The average of the laboratories is globally superior to the target value. With regard to the clinical cases, even if rhesus prophylaxis is rather well advised (70 to 95% of correct answers), the answers to the clinical cases show a heterogeneity of the practices essentially on the additional tests to be carried out (10 at 95% correct answers). However, there is an improvement in the results. The fetal risk was poorly assessed in the first assessments with frequently less than 75% appropriate answer. The fetal risk is now better estimated, increasing from 30% in 2019 to 93% of appropriate answers in 2025. Difficulties persist in the presence of uninterpretable cells (ARBC with HbF = F-cells).

Year	Assessment	KHL FRBC/10000ARBC	Average of labs FRBC/10000ARBC	Acceptable limits +/-	Interlabs variability	% of labs with results out of range	KHS FRBC/10000ARBC	Average of labs FRBC/10000ARBC	Acceptable limits +/-	Interlabs variability	% of labs with results out of range	Number of laboratories participating
2016	1601	22	29	40%	27.3	51	0	NA	≤ 3FRBC	NA	37	45
	1602	104	145	40%	27.7	49	104	116	40%	27.7	33	45
2017	1701	10	14	40%	30	51	97	92	40%	27	28	72
	1702	25	36	40%	28.1	52	50	63	40%	27.4	50	71
2018	1801	450	460	30%	21.6	37	12	10	40%	31.5	37	136
	1802	49	67	40%	26.7	54	12	16	40%	27.5	57	139
2019	1901	256	227	30%	21.5	31	31	30	40%	25.1	30.9	152
	1902	CIN	NA	NA	NA	65	79	88	30%	25.6	46.6	155
2020	2001	600	589	20%	NC	39	0	NA	< 5FRBC	NA	1.8	162
	2002	22	29	40%	21.8	48	12	17	50%	28.7	47	152
2021	2101	63	59	30%	22.5	31	64	75	30%	24.1	43	169
	2102	206	207	30%	17.1	29.8	12	18	50%	25.5	56.6	162
2022	2201	100	106	30%	19.4	28.7	6	8	65%	28.2	25.5	150
	2202	350	340	25%	16.6	26.6	100	105	30%	22.2	29.6	155
2023	2301	30	29	40%	22.1	24.1	50	61	30%	20.2	47.6	166
	2302	60	61	42-78	22.4	25.5	120	148	100-180	24.5	42.5	171
2024	2401	CIN	NA	NA	NA	40.6	7	8	3-14	28.8	14.7	172
	2402	270	268	185-384	18.6	21.4	480	477	385-600	19.2	32.2	168
2025	2501	17	17	10-24	27	22.4	30	33	18-45	25.7	20.1	169
	2502	170	166	125-224	19.7	23.7	24	29	14-44	25.4	12.5	170

NC = Not communicated
NA = Not applicable

% of laboratories that gave correct answers	Assessments																																							
Items	KHL 1601	KHS 1601	KHL 1602	KHS 1602	KHL 1701	KHS 1701	KHL 1702	KHS 1702	KHL 1801	KHS 1801	KHL 1802	KHS 1802	KHL 1901	KHS 1901	KHL 1902	KHS 1902	KHL 2001	KHS 2001	KHL 2002	KHS 2002	KHL 2101	KHS 2101	KHL 2102	KHS 2102	KHL 2201	KHS 2201	KHL 2202	KHS 2202	KHL 2301	KHS 2301	KHL 2302	KHS 2302	KHL 2401	KHS 2401	KHL 2402	KHS 2402	KHL 2501	KHS 2501	KHL 2502	KHS 2502
Risk of fetal anemia	58	100	84	32	80	90	59	8	73	75	41	75	92	73	23	36	90	95	81	83	58	64	88	85	89	92	90	82	76	57	69	95	48	88	94	96	83	81	93	87
Risk of maternal immunization	75	57	96	71	93	89	99	59	96	92	98	92	61	96	39	96	81	39	94	81	96	88	72	90	83	87	83	96	94	97	83	95	77	93	96	95	79	97	96	96
Anti-D prophylaxis	93	68	89	9	94	66	97	71	93	92	99	96	80	97	73	95	80	71	91	61	93	90	84	88	87	89	87	93	90	95	93	99	81	85	94	93	88	97	94	95
Dosage adjustment	91	84	80	9	87	89	75	71	93	83	91	68	/	/	75	85	80	94	/	/	83	82	/	/	/	/	88	85	67	88	93	99	39	89	91	94	93	73	/	/
Control of KBT	58	77	80	43	82	/	88	84	70	49	81	74	76	84	26	90	71	89	69	54	83	64	74	78	88	71	66	96	82	86	88	97	32	74	95	95	81	87	96	84
Control of RBC Antibody screen	79	62	50	38	/	80	52	78	78	80	91	87	/	/	/	/	88	61	92	87	/	/	86	88	89	91	93	95	94	96	90	98	82	94	95	95	87	96	95	95
Emergency transmission to clinician	73	93	98	41	69	97	97	100	/	/	/	/	98	71	/	94	/	/	/	/	/	/	94	54	96	68	72	94	/	/	94	97	/	/	96	96	66	42	96	48
hemoglobin electrophoresis	/	/	/	/	/	/	/	/	/	/	/	/	/	/	69	85	80	96	/	/	/	/	/	/	/	/	/	/	/	/	/	/	74	93	86	85	85	94	91	91
TOTAL	78	77	77	42	87	86	79	71	83	80	86	84	83	86	54	73	81	78	86	73	82	78	83	81	89	83	83	92	85	87	88	98	65	87	93	93	83	85	93	85
															F-cells																		F-cells							
% of laboratories that gave correct answers			[75% - 100%		[50% - 75%[		[25% - 50%[		[0% - 25%[																															

CONCLUSION

In view of these encouraging results, we must now identify more specifically the laboratories for witch the results are systematically out of limits and could generate a risk for patients. The objective of this EQC is to disseminate advice on good practice for carrying out this test. It is also to help biologists to decide on the fetal, neonatal and maternal risk, on rhesus prophylaxis of the RhD negative patient and finally on the additional tests to be carried out (Kleihauer control / Red blood cell antibody screening test / hemoglobin electrophoresis). Since 2016 we have observed an improvement in the management of this test, with less heterogeneity of practices.