Performance of 3 Methods for Quality Control for Gynecologic Cytology Diagnoses

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Objective

To evaluate performance and viability of internal quality control (QC) strategies in a public health laboratory of the state of São Paulo.

Study Design

A retrospective study was performed with 3 QC strategies to improve internal cytologic diagnoses: morphologic guided-list criteria (MGLC), 100% rapid-rescreening (100% RR) of negative slides ("turret" method) and 10% rescreening (10% R) of negative slides. Cases were examined at Adolfo Lutz Institute, São Paulo, Brazil, from 2002 to 2004. Histopathologic results, when available, were considered gold standard; cytologic consensus diagnosis was by 2 pathologists when histologic results were unavailable.

Results

MGLC selected 20.7% samples with cytologic atypias, 10% R selected 0.6% and RR selected 2.5%. Cytologic/histologic initial concordance was 57.4%, low-grade squamous intraepithelial lesion false negative rate was 34.9% and high-grade squamous intraepithelial lesion false negative rate was 12.2%. After diagnosis, consensus concordance was 97.2%.

Conclusion

The 100% RR and 10% R QC strategies detected more false negative cases in liquid-based cytology than in conventional Pap smears. The 100% RR strategy reduced the false negative results and allowed evaluation of individual staff performance. The 10% R strategy did not offer significant results. We concluded that association of MGLC and 100% RR strategies might improve cytologic diagnostic quality. (Acta Cytol 2008;52:0000–0000)

> **100% RR is supposed to have relatively low cost compared with other rescreening modalities, making it an attractive option for internal control in low-resource settings.**

The Pap test is considered an efficient and safe strategic option for the recognition of uterine cervix carcinoma and its precursor lesions because it is well accepted by women and clinicians in countries

where organized or opportunistic screening with Pap test cytology has substantially reduced cervical cancer morbidity and mortality over the last 50 years.[1] Unfortunately, despite the historical benefits, serious criticism has emerged against the Pap test as a screen-

> *Our results confirm the qualities of 100% RR as a useful option for internal quality control of cervical cytology diagnoses and to avoid false negative results.*

ing tool due to a considerable number of false negative results.[2] Indeed, Pap test cytology has failed to reduce cervical cancer mortality in many developing countries, and most low-income countries cannot make the necessary public health investments to deploy organized screening.[1]

Despite the fact that Pap test screening has effectively reduced the incidence and mortality rates of cervical cancer in several countries, these rates still vary greatly. Along with the background risk, variation in the rates depends fundamentally on accessibility and quality of screening.[3]

The quality of Pap test results still remains crucial to maintain the parameters of confidence to refer women with abnormal cytology to colposcopy. It is certain that conventional cytology faces serious questions related to unsatisfactory results or it may be limited for a number of technical reasons.[4] Liquid-based cytology (LBC) has greatly enhanced the quality of cytologic preparations, significantly improving the adequacy of the samples; reducing the artifacts and biases, which limited the cytologic interpretation; and, additionally, preserving material for molecular analyses.[4] However, a principal concern in Pap test results is the extremely high variability of performance rates and categorization of the lesions among cytologists, which occurs regardless of the method used to prepare the samples, whether conventional or liquid-based.[5]

Adolfo Lutz Institute is a reference laboratory of surveillance of cytologic diagnostic quality in the state of São Paulo, Brazil. Our mission in cytology is to review and control cytologic diagnoses and propose and discuss strategies of QC and quality assurance (QA) with laboratories that work in cervical cancer prevention. Moreover, we are constantly stimulating the development of internal systems for diagnostic control in these laboratories.

The objective of this work was to evaluate the performance of 3 methods of internal diagnostic QC (re-

view based on a list of informative and comprehensive criteria, review of 10% of negative cases and 100% of rapid rescreening of negative cases) in order to evaluate the qualities and limitations of each method.

Materials and Methods

We have randomly evaluated 2,774 liquid-based cytology samples and 1,410 conventional Pap smears, examined at the Cytology Laboratory of Pathology Division of Adolfo Lutz Institute, São Paulo between 2002 and 2004. The Pap smear was conventionally collected and fixed in carbowax. LBC was collected and prepared with the DNA-Citoliq Kit (Digene Brasil, São Paulo, Brazil) following the manufacturer's instructions.

The majority of the analyzed cases contained information of previous diagnosis: 3,022 of the cases had presented previous negative cytology, 110 had a previous history of lesion of the uterine cervix, 1,041 had no information available and 11 had no previous cytologic screening.

Informative and Comprehensive Criteria

Primary screening is routinely submitted to a protocol of internal QC composed by a list of informative and comprehensive criteria that select high-risk cases for review; we have named the method morphologic guided-list criteria (MGLC). These criteria include previous cytologic abnormalities, history of viral infection, postmenopausal hemorrhage, unsatisfactory samples, squamous intraepithelial lesions (SILs), glandular-like alterations and visual inspection abnormalities. All selected cases included in this list are routinely fully reviewed by 3 cytologists.

Review of 10% (10% R) of Negative Cases and 100% Rapid Rescreening (100% RR) of Negative Cases

The 10% R and 100% RR was performed without positive (SILs, cancer and atypical squamous cells of undetermined significance [ASCUS]/atypical glandular cells of undetermined significance [AGUS]) or unsatisfactory cases because all of them were reviewed by 3 observers (2 cytologists plus 1 cytopathologist, at least). In this part of the study, all cases categorized as negative for SILs and malignancies were included. In 10% R the cases were rescreened by 2 cytologists with experience and previous training in this type of revision.

For 100% RR, the turret method was used by the 2 observers, who were also experienced with this method following conditions previously tested.[6] The reading time for 100% RR was standardized at 60 seconds, for both conventional cytology and LBC preparations. The review was performed by optical microscopy with a magnification of ×100. When any

suspected alteration was found, the examination was promptly interrupted and the case was submitted for conventional screening.

For the positive or suspicious cases found in both reviews, the gold standard was the histopathologic result; for cases without biopsy results, the gold standard was the consensual diagnosis ascertained by 2 cytopathologists.

Cytology and Histology Classification

Cytologic abnormalities were classified according to the official Brazilian terminology, largely based on the Bethesda terminology.[7] Histologic findings were categorized according to the World Health Organization (WHO) classification.[8]

Statistical Evaluation

The Conquistador System (Continuous Quality Improvement by Statistical Analysis for the diagnostic objective reports; version 1.6.0, November 2004, Superiore Institute di Sanit, Rome, Italy) was used for entering the results and calculating the simple and weighed κ, according to recently published instructions.[9]

Results

A total of 4,184 samples were evaluated with 3 different strategies of internal QC: list of informative and comprehensive criteria and 10% R and 100% RR of the negative cases.

The results of the first screening were: 76.8% negative for the neoplastic cells, 2.5% unsatisfactory and 20.7% with lesions (Table I). From this total, 2,774 of 4,184 (66.3%) were LBC and 1,410 of 4,184 (33.7%) were conventional Pap smear.

From 1,117 of 4,184 (26.7%) samples guided by a list of criteria for revision, 865 of 4,184 (21%) deomonstrated cellular changes from ASCUS or above (ASCUS+).

Table II presents diagnoses distribution after 100%

Table I *Frequency of Results Obtained by Morphologic Guided-List Criteria*

Result (N = 4,184)	N (%)
Negative	146 (3.5)
Unsatisfactory	106 (2.5)
AGC	27 (0.6)
ASCUS	329 (7.9)
LSIL	170 (4.1)
HSIL	330 (7.9)
Carcinoma	8 (0.2)
Adenocarcinoma	1 (0.02)
Total	1,117 (26.7)

AGC = atypical glandular cells.

Table II *Frequency of Results Obtained by 10% R and 100% RR*

Diagnosis	100% RR		10% R	
	No.	%	No.	%
Negative	2,958	96.4	327	10.7
Unsatisfactory	32	1.0	21	0.7
ASC-H	59	1.9	10	0.3
ASCUS	5	0.2	4	0.1
AGC	4	0.1	3	0.09
LSIL	4	0.1	1	0.03
HSIL	5	0.2	4	0.1
No examined	—	—	2,697	88.0
Total	3,067	100.0	3,067	100.0

ASC-H = atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion, AGC = atypical glandular cells..

RR and 10% R random rescreening of the negative cases, respectively, in samples prepared with LBC and conventional methods. Of importance, in 100% RR option, LBC detected more lesions than Pap smear, but Pap smear was more effective for categorizing low-grade squamous intraepithelial lesion (LSIL) lesions (Table III). In 10% R, LBC material was quite superior to Pap smear to define cervical lesions (Table IV). From 3,067 samples submitted to the 100% RR, 297 (9.7%) were selected for more detailed evaluation. Of these, 188 (63.3%) remained negative and in 109 (36.7%) the diagnosis was modified. There were more false negative lesions in liquid-based (29%) than in conventional cytology (18%). We also evaluated the time spent to 100% RR. We used a digital timer to get the minimum and maximum times to perform the examination, register the results and exchange the slides on the microscope. The time for 100% RR varied between 1:45 and 4:15 minutes for conventional Pap smears and 1:30 and 3:45 min for LBC, depending on the degree of difficult in each case.

Table V depicts the correlation between histopathologic results and initial cytologic diagnoses. There was an overall concordance of 57.4% considering the initial diagnostic. Computing the diagnoses obtained after review, the rate of false negative cases were 34.9% for LSIL and 12.2% for high-grade squamous intraepithelial lesion (HSIL). The group of 10% R presented 12 cases with biopsy examination: 7 of them (58.3%) concordant and but 5 (41.7%) discordant (cytologic examination led to undercategorization of these cases).

Table VI exhibits the distribution of the cytologic examination after review. All cytologic cases without biopsy were blindly reviewed, and consensus diagnoses were obtained when necessary. Concordance in the blindly reviewed results was 97.2%. The major discordant results were related to glandular and squamous atypias and unsatisfactory cases.

Table III *Diagnoses Distribution After 100% RR of Negative Cases*

Method	Unsatisfactory	Negative	ASCUS	ASC-H	AGC	LSIL	HSIL	Total
LBC	22 (10.3%)	130 (60.7%)	5 (2.3%)	48 (22.4%)	4 (1.9%)	1 (0.5%)	4 (1.9%)	214 (100.0%)
Pap smear	10 (12.0%)	58 (70.0%)	0	11 (13.2%)	0	3 (3.6%)	1 (1.2%)	83 (100.0%)

ASC-H = atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion, AGC = atypical glandular cells.

The κ index between the observers was remarkable high, presenting similar simple κ and weighed κ of 0.98 (95%CI).

Discussion

The cancer of the uterine cervix remains a serious but avoidable illness. It represents the third most common malignant neoplasm in the female population in Brazil, after breast and skin cancer. The 2006 estimated rate was dramatic: 20 per 100,000 new cases were expected.[8]

It is well known that cytopathologic diagnosis is a subjective approach that may create significant biases for diagnostic categorization and a wide range among observers, which generates difficulties in terms of reproducibility of cytologic classification.[5] However, strategies for enhancing the performance of observers have been constantly suggested. Recently, a web-based format was used to compare the performance of cytotechnologists and pathologists using 77 images with classic and borderline cytologic changes, in order to verify the interobserver reproducibility in classifying cervical cytology images. Experienced cytotechnologists and pathologists performed similarly. Participants achieved higher sensitivity for identifying high-grade squamous lesions than they did for high-grade glandular lesions.[10] This is quite interesting because the greatest number of errors is supposed to occur in the misinterpretation of HSIL and adenocarcinomas.[11] Additionally, a great concern of the College of American Pathologists is to assess a minimum regulatory proficiency test to estimate incorrect negative performance to categorize HSIL. The results of a recent inquiry revealed poor performance of pathologists relative to that of cytotechnologists in both conventional Pap smear and LBC, which may reflect a lack of prescreening of slides or scope of practice issues.[12] The ability to recognize cytologic alterations seems to be closely related to daily screening routine.

The minimum time spent by the pathologists to screen cytologic slides is crucial to define the professional skill to discriminate cervical lesions.[12] The proposition of our study is directly focused in this direction because beyond avoiding false negative results, it is also absolutely necessary to harmonize the concepts of cytologic classification.[13] We recently verified that interlaboratory discussion of positive, suspicious, undetermined atypias and unsatisfactory cases considerably improves cytologist performance.[13] Curiously, one of the most frequent discrepancies we observed in our series was related to unsatisfactory categorization, which is recognized as the major validation failure in proficiency programs.[12] From 1,117 of 4,184 (26.7%) samples guided-selected for review, 865 of 4,184 (21%) have showed cellular changes. This method is obviously anecdotal because the selection of cases largely depends on the list of criteria to be reviewed; regardless the severity of the lesions, this approach is important in avoiding overinterpretation or underinterpretation biases. The most traditional method for QC is the review of 10% of negative cases. This practice is criticized because its efficiency is dismal.[2] Additionally, we can reflect on the ethical approach of this proceeding: why do only 10% of women receive this level of care? What can we offer for the remining 90%? This is a serious concern because of the low cytologic sensitivity.[5] In our series, 10% R was not a promising tool because of its poor efficiency. Most of the increased regulation in the practice of gynecologic cytology is related to the Clinical Laboratory Improvement Amendments of 88 (CLIA 88) proposal. Workload limits and mandated review of 10% of negative cases now exists, but accurately measuring the error rate involves significant effort for very little if any reimbursement.[14] The 10% R can be also analyzed under different perspectives. For laboratories in which the professionals are inexperienced, this modality can serve to measure the evaluation of the

Table IV *Diagnoses Distribution After 10% R of Negative Cases*

Method	Unsatisfactory	Negative	ASCUS	ASC-H	AGC	LSIL	HSIL	Total
LBC	13 (6.6%)	163 (82.3%)	4 (2.0%)	10 (5.1%)	3 (1.5%)	1 (0.5%)	4 (2.0%)	198 (100.0%)
Pap smear	8 (4.7%)	164 (95.3%)	0	0	0	0	0	172 (100.0%)

ASC-H = atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion, AGC = atypical glandular cells.

Table V *Cytohistopathologic Concordant Diagnoses*

Initial diagnosis	Cervicitis	CIN 1	CIN 2	CIN 3	Carcinoma	Total
Negative	97	30	7	3	0	137
Unsatisfactory	9	9	6	3	0	27
ASC-H	2	3	18	18	0	41
ASCUS	0	0	3	2	3	8
AGC	12	0	1	0	1	14
LSIL	22	13	7	4	0	46
HSIL	3	1	0	0	0	4
Total	145	56	42	30	4	277

ASC-H = atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion, CIN = cervical intraepithelial neoplasia.
HSIL comprised CIN2/CIN3.

maturity process, with a senior professional inspecting the performance of new cytologists and determining the degree of trustworthiness of each regarding their capacity for preventing false negative results. It is supposed that 10% R also serves to maintain the attention of the cytologists, because screening is a monotonous, exhaustive and laborious activity.[13]

Another methodologic approach for QC intended to diminish the impact of high false negative rates is rapid prescreening or rescreening. Both methods are believed to be superior to 10% R.[15-19] Our results have supported these previous reports showing that 100% RR identifies more lesions than 10% R, in both LBC and Pap smears. LBC presents many advantages compared to conventional cytology, such as time required for screening, the facilities required for LBC thin layer presentation, a reader-friendly procedure and reduced distraction factors. Additionally, the time required for 100% RR for LBC was always sufficient for LBC but not for Pap smear. Part of the difficulty related to the extension of the smears and crowded appearance of overlapped cells. It is supposed that for routine conditions, conventional screening varies from 6 to 7 minutes, but for LBC is much less.[2]

Efforts to avoid errors in cytology examination should be seriously analyzed. In our series, the 10% R appears less efficient than the other options. Review of part of the routine cases, selected by means morphologic and clinical information, seems to be an efficient option for procedure quality in terms of both educational proposal and to avoid errors of lesion categorization, but it is clearly not appropriate for avoiding false negative interpretations. On the other hand, 100% RR seems to be most effective for this purpose, but may not be realistic for laboratories with a large number of slides per day. What are the cost implications with 100% RR? Rapid review is believed to be advantageous as an internal QA modality, mainly in unscreened high-risk populations. Also, 100% RR is supposed to have relatively low cost compared with other rescreening modalities, making it an attractive option for internal control in low-resource settings.[20]

Our results confirm the qualities of 100% RR as a useful option for internal QC of cervical cytology diagnoses and to avoid false negative results. Further studies addressing costs should be performed to determine the cost-effectiveness of this quality control procedure in a public health cytology laboratory.

Acknowledgments

Table VI *Comparison of Initial and Consensual Diagnoses After Revision*

Initial diagnosis	Negative	Concensual diagnosis						Unsatis-factory	Adeno-carcinoma	Total
		LSIL	HSIL	Carcinoma	ASC-H	ASCUS	AGC			
Negative	2,976	0	0	0	59	5	4	32	0	3,076
LSIL	0	143	0	0	0	0	0	0	0	143
HSIL	0	0	289	0	0	0	0	0	0	289
Carcinoma	0	0	0	0	0	0	0	0	0	0
ASCUS	0	0	0	0	0	283	0	0	0	283
AGC	0	0	0	0	0	0	23	0	0	23
Unsatisfactory	0	0	0	0	0	0	0	92	0	92
Adenocarcinoma	0	0	0	0	0	0	0	0	1	1
Total	2,976	143	289	0	59	288	27	124	1	3,907

ASC-H = atypical squamous cells cannot exclude high-grade squamous intraepithelial lesion, AGC = atypical glandular cells.

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