

# Evaluation of the automated QIASymphony AXpH sample preparation protocol as an alternative to Manual Conversion of PreservCyt® specimens for use in Hybrid Capture® 2 Assay



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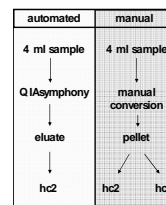
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## Introduction

- Liquid-based cytology specimens such as PreservCyt® (PC) are routinely used for cervical cancer screening by the Papanicolaou test (PAP test, cervical smear, or smear test). Although the Pap test is not very reliable, use of Liquid-based cytology media is known to improve the diagnostic accuracy of PAP smear diagnostic test.
- Diagnostic HPV testing is commonly based on more sensitive DNA-targeted assays such as the commercially available digene High-Risk HPV Hybrid Capture 2 Test® (hc2) or PCR (that uses consensus primer sets PGMY09/PGMY11 or GP5+/6+ to detect most of the HPV subtypes).
- Cervical specimens collected in PreservCyt® (PC) are acceptable for use with the digene hc2 High-Risk HPV DNA Test (hc2). The approved hc2 PC sample conversion protocol requires the manual processing of 4 ml of PC specimen.
- In this research study, we evaluated the feasibility of using the automated QIASymphony® AXpH protocol and kit<sup>†</sup> for purification and concentration of DNA from cervical specimens collected in PC for testing with hc2. This processing method requires no centrifugation and offers increased walk-away time as compared to the manual conversion.

## Study set-up

- 2104 cervical specimens were collected in PreservCyt at Molecular Pathology Laboratory Network (MPLN), Shiel Medical Laboratory (SML) and various sites in Washington, DC from Feb-June, 2009 and tested with the hc2 High-Risk HPV DNA Test. The residual samples were de-identified to comply with patient privacy regulations.
- As reference, 4 ml aliquots of these PreservCyt specimens were processed following the instructions for manual conversion of PC samples
- For evaluation of the fully automated sample preparation, separate 4 ml aliquots of the PreservCyt specimens were processed on the QIASymphony using the AXpH DNA kit.
- The QIASymphony processed specimens and manually converted specimens were tested using the digene hc2 high-risk HPV test and the results compared.



## Methods

- 4 ml of PC sample were placed onto the QS worktable. Cells quickly sediment. In the QIASymphony AXpH DNA protocol 2 ml of sample is aspirated from the bottom of the secondary sample tube. Therefore an increased sample input volume of 4 ml will provide an enriched sample source.
- DNA is extracted using the QIASymphony AXpH DNA Kit and protocol. Cells are lysed, nucleic acid is bound to the magnetic particles, washed and eluted in a final volume of approximately 60 µl.
- Eluates were mixed with 25 µl of DNR2, a modified denaturation buffer freshly prepared from 2 volumes of Buffer N2 and 3 volumes of Buffer D2.
- The denatured eluates were directly used in the digene high risk HPV hc2 test. The obtained RLU/co values were plotted into correlation plots. Concordance analysis with the manual conversion method was performed.

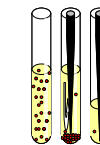
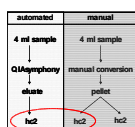


Fig. 1: Cell enrichment by sedimenting

## Comparison: QIASymphony 4 ml versus MC 4 ml



- 1056 specimens were tested at MPLN, 528 specimens at Shiel and 520 specimens were tested at QIAGEN. Cytology data were only available for the specimens tested at QIAGEN.
- Table 1 shows the RLU/co distribution of automated and manual sample conversion by study site.
- Two population types tested: MPLN and Q-R&D: ASCUS+ reflex testing (high rate of positivity) and Shiel: adjunctive screening (high rate of negativity)

| RLU/co Range | MC hc2      |            |            | QIASymphony AXpH hc2 |            |            |
|--------------|-------------|------------|------------|----------------------|------------|------------|
|              | MFLN        | Shiel      | Q-R&D      | MFLN                 | Shiel      | Q-R&D      |
| < 1          | 424 (40%)   | 455 (86%)  | 144 (28%)  | 473 (45%)            | 450 (85%)  | 139 (27%)  |
| 1-2.5        | 58 (5%)     | 18 (3%)    | 23 (4%)    | 47 (4%)              | 21 (4%)    | 26 (5%)    |
| 2.5-10       | 102 (10%)   | 14 (3%)    | 53 (10%)   | 93 (9%)              | 14 (3%)    | 54 (10%)   |
| > 10         | 472 (45%)   | 41 (8%)    | 300 (58%)  | 443 (42%)            | 43 (8%)    | 301 (58%)  |
| Total        | 1056 (100%) | 528 (100%) | 520 (100%) | 1056 (100%)          | 528 (100%) | 520 (100%) |

Table 1: Comparative RLU/co distribution of specimens tested by manual conversion (MC hc2) and QIASymphony AXpH conversion (QIASymphony AXpH hc2) RLU/co.

## Comparison: QIASymphony 4 ml versus MC 4 ml (II)

- No significant difference between methods in screening populations (Shiel) and ASCUS+ populations (Q-R&D).
- MPLN observed a small difference in specimen positivity rate between both methods.
- Overall AXpH sensitivity: 95.6%; overall AXpH specificity: 96.2%
- RLU/co range of discordant samples: <1: 21%; 1-2.5: 38%; 2.5-10: 34%; >10: 7%

| (a) MPLN |     | MC hc2 |      |     |
|----------|-----|--------|------|-----|
| AXpH hc2 |     | pos    | neg  | sum |
|          |     | pos    | 567  | 16  |
| neg      | 23  | 450    | 473  |     |
| sum      | 590 | 466    | 1056 |     |

| (b) Shiel |    | MC hc2 |     |     |
|-----------|----|--------|-----|-----|
| AXpH hc2  |    | pos    | neg | sum |
|           |    | pos    | 64  | 14  |
| neg       | 9  | 441    | 450 |     |
| sum       | 73 | 455    | 528 |     |

| (c) Q-R&D |     | MC hc2 |     |     |
|-----------|-----|--------|-----|-----|
| AXpH hc2  |     | pos    | neg | sum |
|           |     | pos    | 362 | 19  |
| neg       | 14  | 125    | 139 |     |
| sum       | 376 | 144    | 520 |     |

| (d) All Samples |      | MC hc2 |      |     |
|-----------------|------|--------|------|-----|
| AXpH hc2        |      | pos    | neg  | sum |
|                 |      | pos    | 993  | 49  |
| neg             | 46   | 1016   | 1062 |     |
| sum             | 1039 | 1065   | 2104 |     |

Table 2: 2x2 Table of study sites: a) MPLN, b) Shiel, c) Q-R&D, d) all data

## Comparison: QIASymphony 4 ml versus MC 4 ml (III)

- All 2057 QIASymphony AXpH hc RLU/co values plotted against the MC hc2.
- Solid line represents the ideal correlation, the dashed lines represents 2-fold deviation in RLU/co.

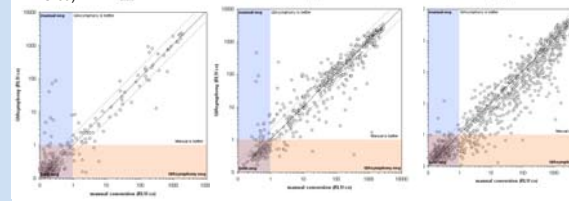
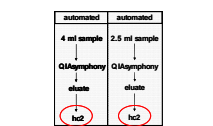
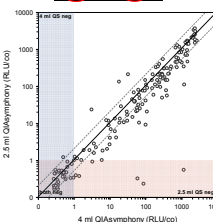


Fig 2: AXpH and MC hc2 RLU/co values plotted. (a) Shiel (b) Q-R&D, (c) MPLN

## Comparison: QIASymphony 4 ml to QIASymphony 2.5 ml



| QIASymphony | Manual Conversion |          | % total agreement |
|-------------|-------------------|----------|-------------------|
|             | positive          | negative |                   |
| positive    | 126               | 2        | 95.2%             |
| negative    | 6                 | 32       |                   |



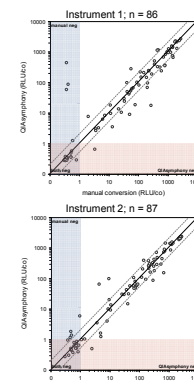
- 2.5 ml aliquots from 166 residual PC samples were placed on the QIASymphony and DNA was extracted from 2 ml enriched fraction using the AXpH DNA Kit and protocol (see "Methods" panel).
- As reference, 4 ml of the same PC samples were processed using the QIASymphony and 2 ml enriched fraction were processed using the AXpH DNA Kit and protocol.
- RLU/co values are plotted in the diagrams (solid line represents the ideal correlation, the dashed lines represents 2-fold deviation in RLU/co).
- Although an agreement of 95.2% is achieved, the RLU/co is slightly reduced with reduced QIASymphony sample loading input volume.

## Instrument to Instrument Variation

| Instrument 1, n = 86 | Manual Conversion |          | % total agreement |
|----------------------|-------------------|----------|-------------------|
|                      | positive          | negative |                   |
| QIASymphony positive | 64                | 4        | 93.0%             |
| QIASymphony negative | 2                 | 16       |                   |

| Instrument 2, n = 87 | Manual Conversion |          | % total agreement |
|----------------------|-------------------|----------|-------------------|
|                      | positive          | negative |                   |
| QIASymphony positive | 65                | 4        | 94.3%             |
| QIASymphony negative | 1                 | 17       |                   |

- Two QIASymphony instruments were used to extract 4 ml aliquots from 86 or 87 residual PC samples, respectively. Two different operators were running the two QIASymphony instruments.
- As reference, 4 ml aliquots of the same PC samples have been manually converted by the two operators.
- RLU/co values are plotted in the diagrams (solid line represents the ideal correlation, the dashed lines represents 2-fold deviation in RLU/co).
- Both instruments provide a comparable performance.



## Conclusion

- The QIASymphony AXpH DNA protocol provides a fully automated sample preparation method for cervical PreservCyt samples.
- A complete set of 96 samples can be processed in less than 4.5 hours.
- The overall total agreement between QIASymphony AXpH and MC was >94%. The negative agreement >95%.

|                        | 95% Confidence interval |                  |
|------------------------|-------------------------|------------------|
|                        | Lower                   | Upper            |
| Positive agreement (%) | 92.51%                  | 93.93%           |
| Negative agreement (%) | 95.90%                  | 96.95%           |
| Total agreement (%)    | 94.16%                  | 95.08%           |
| kappa                  | 0.88                    | 0.90             |
| McNemar                | χ <sup>2</sup>          | p-value = 0.0004 |

- The reduced sample input volume of 2.5 ml results in slightly reduced RLU/co signals in the hc2 assay.