Recent experience of ABO blood discrepancies with aids of ABO genotyping

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【Background】ABO genotyping is essential for the resolution of ABO discrepancy and for the determination of ABO subgroups. Most clinical samples, including suspected inherited subgroups and acquired variant phenotypes, can be determined by PCR-sequencing of exons 6 and 7 in the ABO gene. Here, we described the recent cases of ABO discrepancy with helping of ABO genotyping.

【Methods】We conducted a retrospective investigation of serological and genotypical data requested for ABO discrepancy to the blood bank at Chonnam National University Hospital and Chonnam National University Hwasun Hospital between August 2013 and December 2014. ABO genotyping was performed with PCR-direct sequencing of exons 6 and 7 in the ABO gene; the standard serologic tests were also performed.

【Results】The causes of discrepancy were categorized as follows: extra serum reactivity was the most common cause with 10 cases (37%), and weak/missing red cell reactivity (9/27, 33%), mixed field red cell activity (5/27, 19%), and weak/missing serum reactivity (3/27, 11%) are following (Table 1). Aids of ABO genotyping, 59% (16/27) of ABO discrepancies could be resolved and cis-AB allele was the most common allele (7/16, 44%) which were demonstrated as a cause of ABO discrepancy by ABO genotyping (Table 2). A half of cases (8/16) requested for ABO genotyping was the case with in vitro fertilization – embryo transfer (IVF-ET) twin. Above them, 2 of 8 cases with IVF-ET twin were the blood chimera showing phenotype of A\textsubscript{int}B\textsubscript{3}.

【Conclusion】We ensure that ABO genotyping using PCR-direct sequencing is useful for the resolution of ABO discrepancies and for the investigation of ABO subgroups. ABO genotyping will be also helpful for the evaluation of IVF-ET twin arising as a cause of ABO discrepancy recently.

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Comparison between automated and manual reading for evaluation of BCR/ABL FISH

Introduction
FISH (Fluorescent in situ hybridization) has become an essential tool for diagnosing and monitoring hematological disease. Testing for minimal residual disease requires precise and accurate normal cut-offs. There is no consensus in the field on the correct method of establishing a normal reference range. We discuss and compare automated analysis with manual analysis and settle reference range of cut-offs.

For chronic myeloid leukemia, the FISH detection of t(9;22)(q34;q11) in interphase nuclei is an alternative method to bone marrow karyotyping for monitoring treatment. Automation system suggests the circumvention of several drawbacks of manual analysis. In this study, the capabilities of a commercially available automated image acquisition and analysis system were evaluated by comparison study with manual reading method.

Method
Cell suspension was prepared from peripheral blood of 10 healthy controls. The commercially available BCR/ABL dual color, dual fusion (DF) probe kit (Vysis, Downers Grove, IL) was used for labeling the respective BCR and ABL gene regions. The cells and probes were denatured on a heating plate together at 80°C for 5 min. Hybridization was performed overnight at 37°C. Denaturation and hybridization was performed using Xmatrix system (Abbott, BioGenex, USA).

FISH analysis was performed by using both automated Bioview Duet™ (BioView, Ltd., Billerica, MA) system and manual reading. We also calculated cut-off value using BETAINV function of excel (Microsoft, Redmond, Washington, USA). Analysis of at least 200 nuclei on each preparation was performed.

Result & Conclusion
The cut-off value calculated from Bioview analysis for 3O3G2F pattern was 1.1%, and manual reading result is 1.2%. This result was similar to established cut-off value by the manufacturer. Otherwise, cut-off value calculated from Bioview analysis for 2O2G1F pattern was 18.6%, and manual reading result is 15.7%. The concordance of two analytic method was 96.5%~99.8% in major signal patterns. We suggest that 2O2G1F pattern of FISH for BCR/ABL1 gene rearrangement was observed when 3O3G2F pattern was lower than 1% in normal healthy person.

Our study indicates that Bioview system is a reliable method to analyse FISH for BCR/ABL1 gene rearrangement. And Bioview system represents a clinically useful tool for standardised and objective BCR/ABL1 gene rearrangement of hematologic malignancy patients.
Human Papilloma Virus (HPV) Genotype Screening
by PCR-restriction Fragment Polymorphism Assay

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Background: Uterine cervix cancer is the worldwide most popular cancer which ranks on the second next to breast cancer in women. Human Papilloma Virus (HPV) infection is deeply connected to cervical cancer and the occurrence rate of cervical cancer which is caused by HPV is various depend on its diverse genetic type. For these reason, the importance of HPV genetic screening tests has been on the rise in the laboratory for clinical prognosis or cancer treatments. So far, around 120 types of HPV have been reported according to its genetic sequence identification and half of them are also known that potentially related to the genital infection. HPV 16 and 18 are classified as a high risk group and they are found in most of the cervical cancer lesion.

Materials and methods: The number of samples analyzed in this study was 2,742 and those were collected in the Clinical laboratory of Soon Chun Hyang University Cheonan Hospital from January to July, 2013. Samples were subjected to PCR reaction with upstream and downstream primer set which includes reference sequence of each HPVs. After that, PCR products were treated with particular restriction enzyme to produce the oligomers which contain the reference sequence of respective HPVs. The oligomers were analyzed using Restriction Fragment Mass Ploymorphism (RFMP) method with MALDI-TOF MS. The data distribution was analyzed according to the frequency of positive and unusual data results.

Results: In 772 samples (28%) out of 2,742 samples which were requested to the hospital showed positive result as follows: 191 samples (24%), high risk group; 68 samples (8%), moderate risk group; 432 samples, low risk group; 118 samples (15%), unassigned risk; 37 samples (1%), mixed type. HPV 16 ranked the first majority with 78 cases (9.6%) which was followed by HPV 18 with 31 cases (3.8%), HPV 51 with 25 cases (3.1%) and HPV 52 with 20 cases (2.5%) in descending order. Among the moderate risk group, HPV 66 was 41 cases (5.1%) and HPV 53 was 27 cases (3.3%). HPV 62, 61 and 81 which belong to low risk group showed 109 cases (13.5%), 56 cases (8.4%) and 6.9% respectively. In unassigned risk group, HPV 84 showed 27 cases (3.3%) and others with 70 cases (8.7%).

Conclusion: In the previous studies, HPV infection plays pivotal role in uterine cervical cancer was confirmed. As the infection of HPV progressed on, High risk HPV is increasingly observed in lesion of cervical squamous cells. Analysis of HPV genetic type using MALDI-TOF MS is highly considered as clinical screening test for diagnosis of uterine cervical cancer and cervical squamous cell differentiation.

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