

Recent experience of ABO blood discrepancies with aids of ABO genotyping

©Kyung-Hee Park¹⁾, Kyung-Ah Na¹⁾, Sun-Suk Hwang¹⁾, Min Seok Heo¹⁾, Eun Jeong Won¹⁾
Department of Laboratory Medicine, Chonnam National University Hospital, Gwangju, Korea¹⁾

【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_{int}B₃.

【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.

TEL: 010-3623-1779

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

©Jee-yoon Shin¹⁾、 So-young Choi¹⁾、 Je-hyun Park¹⁾、 Je-sung Lee¹⁾、 Jeong-sung Du¹⁾
Hwasun Chonnam University Hospital¹⁾

[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.

Tel ; 82-61-379-7981

Human Papilloma Virus (HPV) Genotype Screening

by PCR-restriction Fragment Polymorphism Assay

©Pilsang You¹⁾

Clinical laboratory of Soon Chun Hyang University Cheonan Hospital¹⁾

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.

(Clinical laboratory of Soon Chun Hyang University Cheonan Hospital, TEL: +82-104415-4077)

Ophthalmomyiasis Caused by a *Phormia* sp. (Diptera:Calliphoridae) Larva in an Enucleated Patient

©Jae-Soo Kim¹⁾

Clinical Laboratory of Dankook University Hospital¹⁾

Introduction : The most common site of fly larva infestation is skin wounds. However, cases of ophthalmomyiasis have rarely been reported worldwide, accounting for <5% of all cases [1]. Most of the patients with ophthalmomyiasis were reported from tropical countries with low socioeconomic status; however, sporadic cases have occurred in developed countries [2]. Ophthalmomyiasis is classified into the external and the internal type, with the former being more common [3]. External ophthalmomyiasis is a limited infestation of superficial periocular tissues, including conjunctival myiasis. Internal ophthalmomyiasis is characterized by the presence of the larva within the eye, which occurs when dipterous larvae penetrate the conjunctiva and sclera, and migrate into the subretinal space [2]. Symptoms, such as severe eye irritation, redness, foreign body sensation, pain, lacrimation, and swelling of the lids, have been reported in patients with ophthalmomyiasis, but complications, including corneal ulcers and decreased vision, are not uncommon [4].

In Korea, 4 cases of myiasis have been reported [5]; however, myiasis of the eye has never been reported. We present here a case of ophthalmomyiasis caused by *Phormia* sp. larva in a Korean man.

Case Record :

A 50-year old male residing in Cheonan-si, Chungcheongnam-do, the Republic of Korea visited the Department of Ear, Nose, and Throat of Dankook University Hospital on 1 June 2010 for a mass in the right auricular area. The mass was diagnosed as a malignant melanoma with metastatic lymphadenopathy, and wide excision was scheduled. He was a day laborer, and had an enucleation of his right eye due to squamous cell

carcinoma 5 years ago. During hospitalization, foreign body sensation developed in his right eye on 9 June, and close examination revealed a moving fly larva inside the eye cavity (external type). The larva was removed and sent to the Department of Parasitology for analysis. A wide excision was performed on 7 June 2010. The postoperative course was uneventful without any complaints of further myiasis.

Discussion : This is the first report of ophthalmomyiasis, the external type, in Korea. In humans, ophthalmomyiasis is commonly caused by the ovine nasal botfly *Oestrus ovis* [1]. The Russian botfly *Rhinoestrus purpureus* which is found in sheep-farming communities [7] and *Hypoderma* spp., including *H. bovis*, have also been reported to be the cause of ophthalmomyiasis [8]. In addition, ophthalmomyiasis caused by *Cochliomyia hominivorax* larvae has been described in Brazil [9].

In recent years, myiasis has been uncommon in Korea. However, a nasal myiasis was reported last year at Dankook University Hospital [5], and the present case occurred again. Judging from the developmental period of the larval stage of *Phormia* sp. and the warm climate, it could have been possible that he was infested with fly larvae during hospitalization [11,16]. Control of fly populations is needed at Dankook University Hospital, and prevention from myiasis is recommended in patients with open, draining wounds through continuous use of dressings. Since it has been suggested that hospital-acquired myiasis has been under-reported [17], careful observation should be performed on the presence of flies in other hospitals.

(Clinical Laboratory of Dankook University Hospital, TEL: +82-10-6868-0114)